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STRUCTURAL CHANGES FOLLOWING ADMINISTRATION OF QUINACRINE HYDROCHLORIDE

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Despite the widespread use of quinacrine hydrochloride (atabrine dihydrochloride) as a prophylactic and therapeutic agent in antimalarial therapy, there has been until recently little publication of experimental work on its pathologic effects. Hecht [1] noted in cats distinct enteritis after oral administration and hyperemia of the liver and fatty degeneration of the kidney after subcutaneous administration of this drug. Martin, Cominole and Clark [2] in a study of the chronic toxicity in dogs, cats and rabbits described certain nonspecific morphologic changes in the liver, the kidneys and the gastrointestinal tract. Wright and Lillie [3] observed in rats given quinacrine orally pigment cell infiltration of the intestinal mucosa, the lymph nodes, the spleen and the liver, interstitial and exudative monocytic pneumonia, focal myocarditis and myositis and portal and hepatic infarcts. Moderate splenic hemosiderosis and nonferrous pigmentation of the epithelium of the medullary tubules were also seen. Recently Hegsted, McKibbin and Stare [4] fed quinacrine to rats in the diet and noted only a minimum of microscopic pathologic changes. These consisted of the appearance of granules in the Kupffer cells of the liver, in the glomerular epithelium and occasionally the tubular epithelium of the kidneys, in the spleen and in the heart muscle.

The present investigation was part of a more general study of the effects of quinacrine in experimental animals.[5] Since the rat revealed the tissue changes found in the other species, the detailed descriptions are limited to the rat.

MATERIALS AND METHODS

Twenty-five dogs, 20 monkeys, 50 chickens, 20 hamsters, 20 guinea pigs and 20 rabbits were used, together with 400 rats of the Carworth Farms strain weighing 125 to 175 Gm. The animals were maintained on adequate diets, fed ad libitum, and usually were housed in individual cages in an air-conditioned room (temperature, 75 F.; relative humidity, 50 per cent). The quinacrine hydrochloride was administered in a 3 per cent solution through a stomach tube. For more complete details of the experimental procedures, see Molitor and co-workers.[5]

Generally the tissues were fixed in a 4 per cent solution of formaldehyde and stained with Delafield's hematoxylin and eosin. Whenever indicated, tissues were preserved in various other fixatives, and differential stains were employed.[6]

For comparative purposes the doses are expressed in percentages of a dose known as lethal dose 50, a dose which will kill 50 per cent of the animals. The LD50 of quinacrine for rats is 900 mg. per kilogram. The therapeutic dose for malaria in man is 0.4 Gm., given daily for one week, and the prophylactic dose is approximately 0.2 Gm., given semiweekly.[7]

MORPHOLOGIC CHANGES IN RATS FED A SINGLE DOSE

Gross Pathologic Changes.—After a single LD50 (i. e., a 100 per cent dose) most of the rats died in four to seven days. Some died in a few hours; others, after ten days. The anatomic changes in the rats which died were more marked than those in the rats which were killed on the same day. The earliest changes were pulmonary and hepatic congestion and a slight yellow discoloration of all the tissues, particularly the lymph nodes. The longer a rat survived, the greater was the degree of yellow discoloration, of loss of weight, of loss of fat from the fat depots and of dehydration. These changes reached a peak in about one week.

One day after the administration of this dose of quinacrine the liver exhibited some flabbiness and accentuation of the lobular markings. After two days large, irregularly shaped and poorly demarcated homogeneous grayish yellow areas of necrosis began to appear mainly in the right lobe and the right half of the median lobe of the liver. Five days later these areas had become well demarcated, were almost always subcapsular and frequently had thin hemorrhagic rims. They tended to be triangular in shape with the base at the surface. The remainder of the liver showed

From the Merck Institute for Therapeutic Research.

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1. Hecht, G.: Arch. f. exper. Path. u. Pharmakol. **170**:328, 1933.

2. Martin, S. J.; Cominole, B., and Clark, B. B.: J. Pharmacol. & Exper. Therap. **65**:156, 1939.

3. Wright, C. I., and Lillie, R. D.: Pub. Health Rep. **58**:1242, 1943.

4. Hegsted, D. M.; McKibbin, J. M., and Stare, F. J.: J. Nutrition **27**:141, 1944.

5. Molitor, H., and others: To be published.

6. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

7. Goodman, L., and Gilman, A.: Pharmacological Basis of Therapeutics, New York, The Macmillan Company, 1941.

prominent lobular markings. The longer an animal survived, the larger was the area of necrosis. After five to seven days a single LD50 resulted in necrosis of approximately one quarter of the entire liver. Such necrotic areas appeared only after single doses greater than 20 per cent LD50.

Five days after the single LD50 the renal medulla was slightly yellow. The coloration was most intense on the tenth day. Occasionally the adrenal glands were slightly enlarged and brownish yellow. At times the stomach revealed several small superficial ulcers of the glandular mucosa or minute black foci with no clearly discernible underlying lesion. The lymph nodes of the small intestine, the axillas, the peripancreatic region and the lateral aortic region were made somewhat prominent by their yellow color. This color also caused the pineal gland and the anterior lobe of the pituitary gland to be conspicuous. The ventricles of the heart were always markedly contracted in the rats which died.

Histologic Changes.—(*a*) Basophilic Substance: Following the administration of quinacrine, a basophilic substance appeared in the cells of the reticuloendothelial system as well as in certain parenchymatous cells. The latter included cardiac and voluntary muscle fibers, liver cord cells, cells of the fascicular zone of the cortex of the adrenal gland and epithelial cells of the nephron. This basophilic substance was best visualized with the Giemsa or the eosin–methylene blue stain following fixation in Helly's fluid.[8] It usually appeared as globules in the cytoplasm of the phagocytic cells and as fine granules or basophilic tinting of the cytoplasm of the other cells. That this substance is quinacrine or closely related to it is indicated by the following observations:

1. Following an intraperitoneal injection of a suspension of quinacrine into rats and mice, basophilic inclusions were demonstrated in the phagocytes of the exudate.

2. Quinacrine in human blood has been shown to localize in the white cells.[9] We have found basophilic inclusions in the lymphocytes of all the nine species of mammals and birds to which large doses were fed.[10] After relatively large and repeated doses the inclusions appeared in the red cells of rats, mice and hamsters.

3. As the yellow color of the juxtacortical region of the renal medulla increased, there was increasing basophilia of the epithelial cells of the loops of Henle and the collecting tubules in the region. There was definitely more of the basophilic substance in the medulla than in the cortex. Chemical analyses revealed more quinacrine in the former than in the latter.[2]

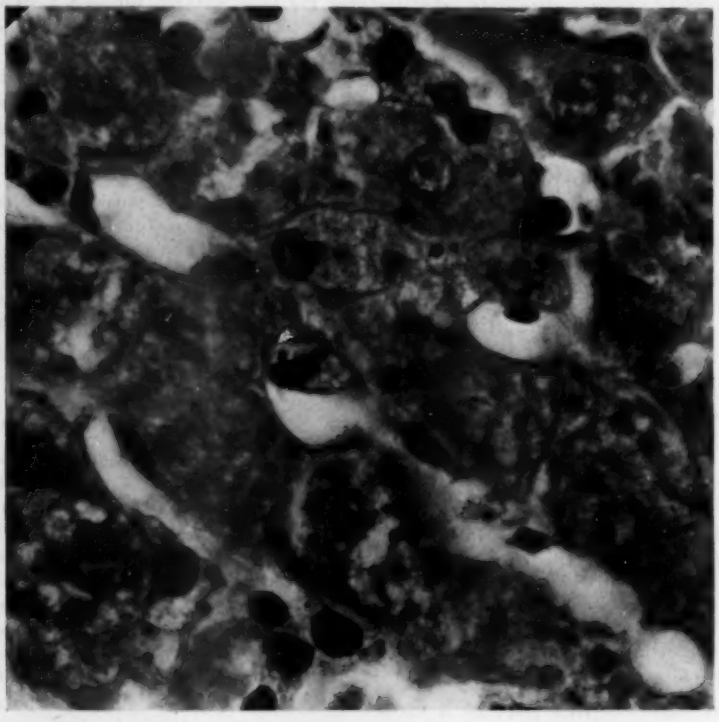

Fig. 1.—Changes in the liver of a rat following administration of quinacrine hydrochloride. Eight doses of 17.5 per cent of the lethal dose killing 50 per cent of rats (LD50) were given by mouth at the rate of three doses a week. The markedly hypertrophied Kupffer cells show vacuolated cytoplasm containing basophilic inclusions. The hypertrophied hepatic cord cells show thinned and coarsened cytoplasm. Eosin–methylene blue stain; × 925.

The differential staining characteristics of the basophilic substance were studied. With the hematoxylin-eosin stain most of the cytoplasmic inclusions in the Kupffer cells were bluish gray and had thin dark rims (fig. 1). They averaged 1 to 3 microns in diameter. The Giemsa and eosin–methylene blue stains imparted a uniform basophilic color to the globules and brought out other smaller bodies which were barely dis-

8. Helly's fluid is a modification of Zenker's solution in which, instead of glacial acetic acid, solution of formaldehyde U. S. P. is added in the concentration of 5 per cent.

9. Shannon, J.: Personal communication to the authors.

10. Mushett, C. W., and Siegel, H.: To be published.

tinguishable with the other stains. The Mallory trichrome stain colored some of the inclusions solid red. Occasional bodies were green but most of them were light brown with thin dark rims. With this stain the nucleoli of the hepatic cord cells were solid dark red, the erythrocytes were green and the lobules of the polymorphonuclear leukocytes were light brown. The inclusions gave negative reactions with the iron stain and with Graham's oxidase stain. With the Mallory basic fuchsin stain all the bodies were light brown with thin dark rims. In this staining quality they resembled the nucleoli of the hepatic cord cells. The red blood cells were gray with a faint brown tint, and the lobules of the polymorphonuclear leukocytes were blue and occasionally dark brown with thick dark rims. Feulgen's stain colored the bodies reddish violet similar to the stain of chromatin. Some of them were a solid color and others had a darker rim.

(*b*) Liver: The large gray areas in the right half of the organ proved to be regions of massive coagulation necrosis. In general there was little inflammatory reaction, but at times the necrotic zones were rimmed with polymorphonuclear leukocytes and often with red cells. Thrombosis of the portal vein or its branches was noted only within or adjacent to the necrotic areas. Serial sections of these thrombosed veins revealed degenerative changes only in the portions of the wall adjacent to the necrotic areas. A slight reaction in the underlying endothelium was discernible.

The earliest changes following a single dose of 25 per cent LD50 occurred in five hours. The portal vein was moderately engorged, the central vein slightly congested and the Kupffer cells slightly hypertrophied. On the second day the cytoplasm of the Kupffer cells showed fine vacuolation and contained several basophilic globules. Ingested white blood cells were occasionally observed. At this time the hepatic cord cells began to show cytoplasmic changes. At the height of the reaction, at seven days, there was a moderate degree of hypertrophy of the hepatic cord cells associated with thinning, coarsening and vacuolation of the cytoplasm. This was associated with slight to moderate hypertrophy of the Kupffer cells. The cytoplasm of these cells was somewhat basophilic, foamy or vacuolated and contained the basophilic inclusions.

With a larger single dose or multiple smaller doses the changes became more marked. Other than the large areas of necrosis there were occasional microscopic foci of necrosis throughout the other lobes. There were many white blood cells mainly congregated in the pericentral areas of the sinusoids. The sudan IV stain revealed an increased amount of sudanophilic material but did not account entirely for the degree of vacuolation observed with the hematoxylin-eosin stain. After repeated large doses fine basophilic granules began to appear in the hepatic cord cells. The nuclei of these cells showed some clumping of the chromatin, and the nucleoli became more prominent. Binucleated Kupffer cells, which are occasionally observed in normal rats, increased in number with the larger doses. Mitotic figures were not observed.

In recovery experiments those animals which survived a single LD50 were killed after four to eight weeks. At this time the rats were well developed, well nourished and well hydrated. Approximately one third of them had in the liver large depressed stellate scars which were firmly adherent to the adjacent structures, including the diaphragm, the duodenum, the greater omentum, the stomach, the right kidney, and the loops of small bowel. These scars extended for a depth of several millimeters into the substance of the liver and were limited mainly to the right half. They proved to be areas of necrotic hepatic cord cells surrounded by a thin fibrous capsule containing small numbers of yellow pigment-ladened macrophages (fig. 2). Some of the pigment took a positive stain for iron. Two weeks after a single dose of 25 per cent LD50 the changes in the cells of the hepatic cords, which were maximal at one week, had disappeared. The hypertrophy of the Kupffer cells and the number of cytoplasmic inclusions were maximal at one week and absent after three weeks.

(*c*) Heart: After a single LD50 the myocardium showed many foci of change, ranging from those of hyaline necrosis of the muscle fibers to foci consisting of necrotic fibers and fibroblasts with occasional lymphocytes and polymorphonuclear leukocytes. In the rats which survived, these foci went on to form focal or diffuse areas of myofibrosis, and occasionally there was revealed an area of calcification and multinucleated giant cells. These changes were most marked in the wall of the left ventricle and toward the base of the heart. Control rats occasionally showed a small myocardial focus of lymphocytes and mononuclear cells. These foci tended to be perivascular and were usually associated with a chronic infection of the lung.

(*d*) Adrenal Gland: Five days after a single LD50 the adrenal glands were yellow and slightly enlarged. The reticular layer (or zone) was congested, while the fascicular layer was widened and composed of hypertrophied and vacuolated cells containing fine basophilic granules. With smaller single doses the only changes were increased compactness and eosinophilia of the cytoplasm and increased vesicularity of the nuclei in some of the cells of the fascicular layer. These changes were not seen in animals killed after one month.

(*e*) Kidney: Five days after a single LD50 the kidney was congested. There was a moderate degree of cloudy swelling, with occasional areas of necrosis of the epithelium of the convoluted tubules and there were basophilic granules in the epithelial cells of the outer segment of the medulla, mainly in the loops of Henle. These granules tended to concentrate toward the free border of the cell. After very large single doses brown droplets were present in the epithelial cells of the proximal convoluted tubules. Some of these stained like hemoglobin, some stained brown with Mallory's basic fuchsin, but all gave a negative reaction with the iron stain. Occasionally hemoglobin casts filled the lumens. Six weeks after 1 LD50 the medullas were still yellow but histologically appeared normal.

(*f*) Spleen: Within one day after a single dose of 25 per cent LD50 there was congestion of the red pulp associated with an increase in polymorphonuclear leukocytes. The congestion and polynucleosis subsided in two days and were followed by an anemia of the red pulp and a decrease in the number of megakaryocytes. Following single doses above 25 per cent LD50, large macrophages containing the basophilic globules and vacuoles appeared in the red pulp. These macrophages disappeared in two weeks. A small amount of brown intracellular and extracellular pigment, which took a stain for iron, was found in all animals, including the controls.

(*g*) Testis: At the higher doses necrotic tubules were infrequently observed. Such tubules were not present in the controls.

(*h*) Lymph Nodes: Many of the nodes revealed fine basophilic inclusions in the endothelial cells and coarser inclusions in the large macrophages and in some of the lymphocytes. The brown pigment found in the macrophages within the sinuses of some of the nodes, especially the peripancreatic, which took a stain for iron, was also observed in the control animals.

(*i*) Stomach: The ulcers which were observed grossly proved to be acute, nonspecific and superficial. The black foci were areas of necrosis in the mucosa with no cellular reaction. The latter were probably terminal phenomena.

(*j*) Bone Marrow: Following the larger doses, there was an increase in the number of segmented and nonsegmented neutrophils. Reticuloendothelial cells containing vacuoles and basophilic inclusions were occasionally found.

(*k*) Striated Muscle: In rats receiving very large single doses an occasional group of fibers in the muscles of the thigh revealed hyaline necrosis and vacuolation.

(*l*) Other Tissues: Basophilic granules were evident in the endothelial cells of the anterior lobe of the pituitary gland and, to a lesser degree, in those of the posterior lobe. There was slight atrophy of the thymus. No other tissue changes that could be attributed directly or indirectly to quinacrine were observed.

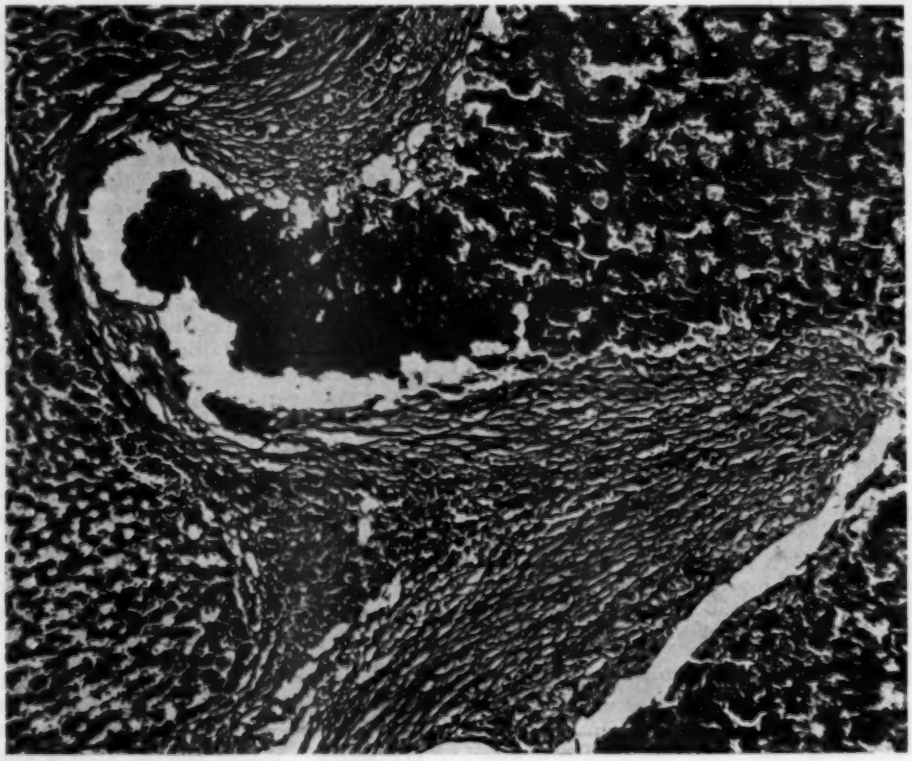

Fig. 2.—Liver of rat killed one month after a single LD50. The area of necrosis is walled off by a thin fibrous capsule. Hematoxylin–eosin stain; × 115.

MORPHOLOGIC CHANGES IN RATS FED MULTIPLE DOSES

The animals receiving daily doses of 25 per cent LD50 died in from five to eight days; those receiving 10 per cent LD50 died in from two to four weeks, and those receiving 5 per cent LD50 died after six to twelve weeks. The degree of the morphologic changes depended on the time of survival as well as on the dose level. Since quinacrine is eliminated slowly,[11] many of the changes are probably dependent on cumulative effects of the drug. The longer the rats lived the more emaciated, unkempt, dehydrated and infested with lice they became. These changes were marked after eight weeks of daily dosing with 5 per cent LD50. With time the paws, the ears, the eyelids and the scleras became progressively yellow.

The area of necrosis in the liver increased and spread from the right lobe to the median lobe and then to the left lobe. The degree of necrosis was proportional to the daily dose and to the period of dosing. After six weeks, three quarters of the liver was composed of large confluent areas of necrotic tissue. The liver became markedly altered in shape and adherent to the surrounding structures. Thin fibrous capsules partially walled off the necrotic areas. The caudate lobe became markedly enlarged as the size of the necrotic area increased. The hepatic cord cells of this lobe were hypertrophied and the cytoplasm vacuolated and somewhat basophilic. Throughout the remaining viable liver tissue there were microscopic foci of necrosis with no cellular reaction and a tendency toward pericentral distribution. The hypertrophied Kupffer cells, which contained a few brown inclusions among the basophilic globules, were increased in number as a result of hyperplasia or concentration. Mitotic figures were not observed. In some areas these cells were detached from the sinusoidal wall and were found lying in the lumens of the sinusoids and central veins. There was a concentration of these macrophages in the pericentral region.

The earliest lesion in the heart could be observed in approximately 20 per cent of the rats after five daily oral doses of 17.5 per cent LD50 on an empty stomach. After nine doses the lesion was marked in almost all the animals. At first basophilic granules appeared at each pole of the nucleus of the normal fibers. Then some of the fibers underwent Zenker's degeneration. Fine vacuoles appeared throughout the cytoplasm of

11. Dawson, W. T.; Gingrich, W., and Hollar, E. D.: Am. J. Trop. Med. **15**:515, 1935.

these fibers, and the basophilic granules appeared to be concentrated within them. The degenerated fibers disappeared, and there resulted a proliferation of fibroblasts with a slight infiltration of lymphocytes and histiocytes (fig. 3). Some of the fibroblasts, unlike the histiocytes, contained only minute amounts of the basophilic granules. With time, these fibroblasts laid down collagen. Except for a slight increase in compactness, the replacement fibrosis which resulted was found to be unchanged eight months after the dosing was stopped. At this time, atrophic and hypertrophic muscle fibers were present within the fibrotic areas, and an occasional fiber revealed hyaline necrosis and vacuolation.

Of the 10 control rats which were killed at various intervals during the test period of fifteen months, 1 revealed a moderate degree of myofibrosis somewhat similar to that following the administration of quinacrine. The myocardium was moderately involved in almost all the rats receiving daily doses of 25 per cent LD50 for one week, 10 per cent LD50 daily for two to three weeks, 5 per cent LD50 daily for ten weeks or 2 per cent LD50 daily for six months. Only a few rats receiving 1 per cent LD50 daily for twelve to fifteen months revealed a slight degree of myocardial involvement.

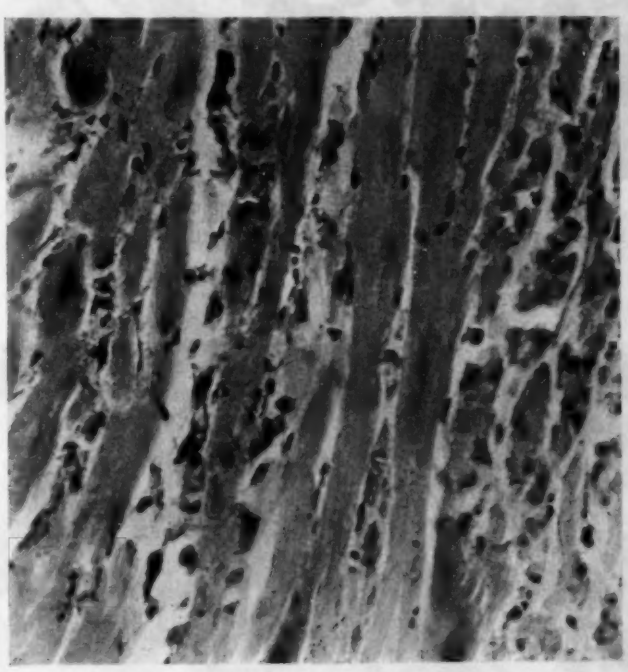

Fig. 3.—Necrosis and replacement fibrosis of cardiac muscle fibers of a rat following nine daily doses of quinacrine administered by mouth, each dose amounting to 17.5 per cent LD50. Eosin-methylene blue stain; × 525.

The muscles of the thigh, those of the pectoral region and the superficial muscles of the back were studied. The lesion, which consisted of necrosis of small groups of fibers with fibroblastic proliferation and slight lymphocytic infiltration, resembled that found in the myocardium. It was present somewhat less frequently and to a lesser degree than the lesion in the heart of the same animal. The muscles of the thigh were those most frequently involved.

The adrenal glands of rats receiving 5 per cent LD50 gradually increased in size and weight and after six weeks were two to three times the normal. At the same time the ratio of adrenal gland to body weight showed a marked increase. The glands were yellowed. The glomerular zone became narrowed and the reticular and fascicular zones were indistinguishable from each other. Both zones were composed of markedly hypertrophied cells with a foamlike or granular cytoplasm and occasionally contained fine basophilic granules.

In the normal adrenal glands most of the sudanophilic material and cholesterol and its esters were present in the glomerular and the outer half of the fascicular zone. Between them was a thin clear zone. After administration of quinacrine the sudanophilic material was lost from the outer part of the fascicular zone and then from the glomerular zone. After nine doses of 17.5 per cent LD50 (three doses a week for three weeks) only an occasional cell in the glomerular zone retained its fat and only a slight amount was discernible in the fascicular zone. The disappearance of cholesterol or its esters as measured by the method of Schultz paralleled the disappearance of the sudanophilic material.

In rats killed after two weeks of dosing, the kidneys showed brown droplets that took neither an iron nor a hemoglobin stain. Cloudy swelling of the epithelial cells of the proximal convoluted tubules was also evident. With longer periods of dosing and larger doses a necrotizing and occasionally a desquamative lesion of the convoluted tubules developed. The amount of basophilic substance began to parallel the degree of yellow discoloration of the medulla. The endothelial cells of the medullary capillaries and the epithelial cells of the glomeruli became hypertrophied, and their cytoplasm became granular and basophilic. After six weeks of daily dosing with 5 per cent LD50, the cytoplasm of the glomerular epithelial cells was slightly to moderately hypertrophied (fig. 4). Massive hemoglobinuria could be induced only in young rats receiving 10 per cent LD50 for about two weeks.[5] Hemoglobin droplets were present in cells of the proximal convoluted tubules, and hemoglobin casts filled some of their lumens (fig. 5). The development of the casts from the droplets was traced.

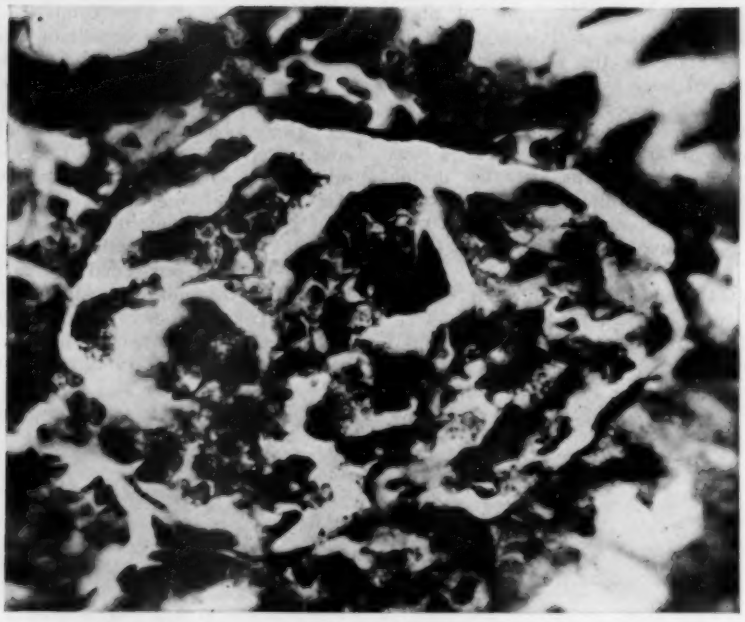

Fig. 4.—Hypertrophied glomerular epithelial cells in a rat fed daily doses of quinacrine for six weeks, each dose amounting to 5 per cent LD50. Hematoxylin–eosin stain; × 700.

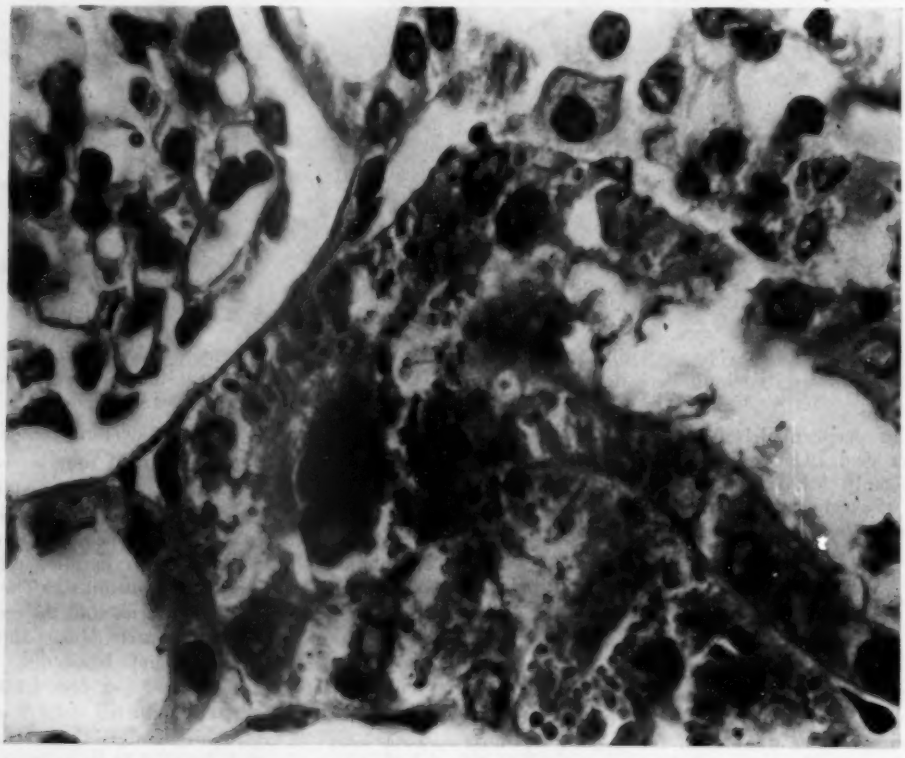

Fig. 5.—Hemoglobin cast in the lumen and hemoglobin droplets in the epithelial cells of a proximal convoluted tubule. From the kidney of a young rat given ten daily doses of quinacrine by mouth, each dose amounting to 10 per cent LD50. Hematoxylin-eosin stain; × 1116.

The spleen increased slightly in size. There was progressive accumulation of macrophages. The lymphoid cells about the trabeculae proliferated and encroached on the red pulp so that after six weeks of daily dosing with 5 per cent LD50 a homogeneity of appearance was evident. There was little differentiation between the red and the white pulp. Slight phagocytosis of red and white blood cells could be seen. The endothelial cells of the venous sinuses and the sheathed arterioles were slightly hypertrophied and basophilic.

Similarly after six weeks the cytoplasm of the endothelial cells of the small arteries and veins of the lung were hypertrophied, finely granular and basophilic. Within the capillaries of the alveolar walls large macrophages, which resembled the detached Kupffer cells, were occasionally present.

Focal areas of necrosis with little cellular reaction were observed in the glandular mucosa of the stomach in many rats which died. At times acute catarrhal enteritis and colitis were observed.

Those animals which received less than 5 per cent LD50 daily were alive after four months. Of the few that were killed, only the rats receiving 2 per cent LD50 revealed changes. The histologic alterations were similar to those previously described and were present to a moderate degree in the kidney and to a slight degree in the adrenal glands, the Kupffer cells and the myocardium. It was only after seven months that brown inclusions, which did not take the stains for iron and hemoglobin, began to appear in the Kupffer cells of the rats receiving 2 per cent LD50.

After five to fifteen months of daily dosing all the rats on the 2 per cent level had died of natural causes, mainly infections of the lungs. None revealed necrosis of the liver. Fifty per cent of the rats receiving 1 per cent LD50 died within the year of infections of the lungs. This signifies little in view of the 30 per cent mortality from similar causes among the controls. Although 20 rats had originally been set out at each dose level, there were too few to permit statistically significant conclusions. Whether quinacrine affects the susceptibility of an animal to natural infections is yet to be established.

The only constant changes in rats receiving 1 per cent LD50 after fifteen months were the slight adrenal and myocardial alterations.

PATHOLOGIC CHANGES IN OTHER SPECIES

The tissues of other species dosed daily with quinacrine were examined. They included tissues of dogs which had received as much as 50 mg. per kilogram for eight months, tissues of monkeys which had received 200 mg. per kilogram for two weeks and others dosed with 40 mg. per kilogram for six months, and tissues of hamsters, guinea pigs and rabbits which had received 90 mg. per kilogram for one to two weeks. The myocardial lesions were observed only in the hamsters. The necrotic lesions of the liver were present in only 1 dog, which had received 100 mg. per kilogram for two weeks and which had not vomited any dose. The changes in the hepatic cord cells and in the reticuloendothelial system were present in all species. The macrophages revealed the basophilic globules in the cytoplasm. Often, however, brown globules, which did not take the iron stain, predominated. These brown inclusions were present after the first dose, while in the rat they appeared only after weeks of dosing.

COMMENT

Pathologic changes noted in rats dosed with quinacrine were present in the heart, the liver, the adrenal glands, the kidneys, the spleen, the reticuloendothelial system and the stomach. The myocardial lesions can be directly attributed to quinacrine. Since the basophilic substance is probably quinacrine, the "toxic agent" causing the degenerative changes in the myocardial fibers has been demonstrated within the cell where it produces its damage. Whether the myocardial changes are the result of a greater susceptibility of the muscle fibers to quinacrine or are due to a greater concentration of the drug is yet to be demonstrated. Focal areas of necrosis of the myocardial fibers have been reported in rats on a diet [12] deficient in potassium, as well as in rats under several other experimental conditions. That the myocardial lesions described here might be related to the changes in the adrenal glands through an altered metabolism of potassium must be considered.

Although the necrotic areas in the right half of the liver resembled infarcts, the only thrombosed veins or venules observed were those within or adjacent to these areas. It could not be established that thrombosis preceded the formation of these infarct-like areas of necrosis. Preliminary experiments with the injection of single doses of quinacrine into the portal or mesenteric veins were not successful in producing necrosis of the liver. This would leave only two possible factors to account for the development of the lesion on the right side, a greater concentration of quinacrine or a greater susceptibility of the cells. A greater concentration might result because of the venous drainage from the gastrointestinal tract into the right side. An analysis of the right and left sides of the liver for their quinacrine content at various dose levels and at various intervals of time after dosing did not reveal any differences. This would indicate that the necrosis is due to a greater susceptibility to quinacrine of the cells on the right side as compared with those on the left.

The components of the reticuloendothelial system in many organs were involved. The changes common to all the cells of this system were the hypertrophy and the basophilic staining of the cytoplasm and the presence of basophilic inclusions. In the early stages of dosing only the Kupffer cells and the macrophages of the spleen were affected. With increasing amounts and longer periods the lymphocytes of the blood [10] and lymph nodes, then the macrophages of the lymph nodes and marrow and finally some of the endothelial cells in various tissues were involved. A similar change was observed in the epithelial cells of different parts of the nephron.

12. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: Am. J. Path. **18**:29, 1942.

The sites where the basophilic substance accumulated might well be where quinacrine localized. The drug would first encounter the phagocytic cells of the body, namely, the reticuloendothelial system. It would then tend to concentrate in the epithelial cells of the kidney which are constantly in contact with it while it is being excreted. That substances may pass from the lumens into the epithelial cells of the kidney has been demonstrated by Smetana and Johnson.[13] The greatest amount of the basophilic substance was found in the epithelial cells of the loops of Henle, the point in the nephron where water is resorbed.

The presence of the basophilic substance in parenchymatous cells was usually associated with pathologic changes. This relationship held for the heart, the liver and the adrenal glands but not for the kidneys. It is of interest that the renal epithelium, which is usually susceptible to "toxins," showed little evidence of damage.

Lesions of the stomach, although frequently present, were not constant and can probably be attributed to the local action of the drug. Trauma as a result of the insertion of a stomach tube was not a factor since similar changes were not found in rats treated in the same way with other substances.

The activation of a latent infection with Bartonella muris cannot account for any of the changes in tissues or cells. The quinacrine-dosed rats did not present Bartonella muris inclusions in the red cells, large amounts of hemosiderin in the Kupffer cells or degenerative changes in the spleen. These changes are characteristic of Bartonella-infected rats.[14]

Despite the great degree of hepatic damage, there was no evidence of a hemorrhagic diathesis in any of the rats.

SUMMARY

The pathologic alterations definitely attributed to quinacrine are the large necrotic foci in the right side of the liver, the necrosis and fibrosis of the myocardium and voluntary muscle and the changes in the cortex of the adrenal gland, in the reticuloendothelial system and in the kidney. Only the changes in the adrenal gland, the kidney and the reticuloendothelial system are reversible.

The basophilic substance found within certain cells of quinacrine-dosed rats is probably quinacrine or a substance closely related to it.

Of changes in dogs, monkeys, chickens, hamsters, guinea pigs and rabbits, myocardial lesions were found only in hamsters. Only an occasional dog revealed necrosis of the liver. Changes in the hepatic cord cells and in the reticuloendothelial system similar to those found in the rat were present in all species.

13. Smetana, H., and Johnson, F. R.: Am. J. Path. **18**:1029, 1942.

14. Gottesman, J. M., and Perla, D.: J. Exper. Med. **52**:121, 1930.

ABSORPTION OF VITAMIN A IN THE RAT

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The recent concept of conditioned or endogenous vitamin A deficiency, which develops despite adequate intake,[1] has given a new significance to the absorption of vitamin A. Investigations have shown that only a small part of administered vitamin A is accounted for in the organism.[2] This fact can best be explained by incomplete absorption of vitamin A in the intestine. However, little information is available as to the excretion of vitamin A in the feces, chiefly because of technical difficulties. Only a few papers concerning the excretion of vitamin A have come to our attention. While vitamin A was found absent from the feces of human subjects on normal diets, after administration of 76,000 units of vitamin A only a small fraction could be found in the feces.[3] Similar results were obtained in rats.[4]

In recent years vitamin A has been visualized by fluorescence microscopy,[5] and this method has been applied to the demonstration of vitamin A in the intestinal wall.[6] No vitamin A has been found in the intestine of the starving rat. However, after feeding this vitamin in oily solution, vitamin A fluorescence was demonstrated in the epithelium and the lamina propria of the villi and in the lacteals of the wall of the small intestine. This picture was considered as an indication of lymphatic absorption of vitamin A and as confirmation of previous experiments on a patient with a fistula of the thoracic duct.[7] Since some other studies on absorption of vitamin A were chiefly concerned with the chemical status in which vitamin A was absorbed,[8] a more extensive histologic examination of the absorption of vitamin A was undertaken in an attempt to investigate conditions and limits of the absorption of vitamin A, to compare the absorption of this vitamin with that of fat and to study the influence of drugs on it. An investigation of the intestinal content for fluorescent vitamin A was included, although the reliabilty and the specificity of the demonstration of vitamin A in the feces by fluorescence microscopy have not been studied as yet.

MATERIAL AND METHODS

Rats weighing approximately 300 Gm. were fed 25,000 to 400,000 U. S. P. units of vitamin A in 0.1 to 0.5 cc. of a natural ester concentrate.[9] The rats were killed at different intervals after the administration of the vitamin. Sections from the wall of the gastrointestinal tract at ten different levels were fixed in solution of formaldehyde U. S. P. (diluted 1:10). Smears of the intestinal content were made at the same levels. Frozen sections of the tissues and the smears were examined under the fluorescence microscope before and after staining with phosphin 3R. The latter is a water-soluble sensitive fluorescent stain which visualizes neutral fat.[10] The unstained specimens were examined for the presence of vitamin A, which is recognized by a green, quickly fading fluorescence. The details of the method are discussed in a previous publication.[10a]

RESULTS

Control Studies of the Intestinal Wall and Content Before and After Feeding Fats Free of Vitamin A.—In a study of 10 fasting rats the wall and the content

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9. This is distilled from fish liver and vegetable oil and contains natural ester in amounts of 200,000 U. S. P. units per gram. It was supplied by Distillation Products, Inc., Rochester, N. Y.

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of the intestine examined at different levels failed to reveal any vitamin A fluorescence. By staining with phosphin 3R a moderate amount of fat could be demonstrated in the wall of the small intestine, beginning in the lower part of the duodenum and extending down to the ileocecal valve. No fat could be found in the large intestine. In the intestinal content some fat was seen all the way down to the rectum.

Two animals received 0.5 cc. of lard liquefied by heating and 0.25 cc. of corn oil, respectively. The lumen and the wall of the intestine were examined for fat, and more was demonstrable in these than in starving rats. However, no vitamin A fluorescence was seen.

Vitamin A Fluorescence of Content and Wall of Intestine in Rats Killed at Different Intervals After Feeding of Vitamin A

Rats Examined	Units of Vitamin A Administered	Lapse of Time Before Rats Were Killed	Site of Vitamin A in Intestinal Content	Site of Vitamin A in Intestinal Wall
2	25,000	15 min.	Stomach and upper part of duodenum	—
2	25,000	25 min.	Stomach, duodenum, beginning of small intestine	Smallest amount of vitamin A in upper part of duodenum
2	25,000	1 hr.	Stomach, duodenum and upper third of small intestine	Duodenum and proximal half of small intestine
2	25,000	3 hr.	Stomach, duodenum and beginning of small intestine	Duodenum and proximal half of small intestine
2	25,000	6 hr.	Stomach and duodenum	Duodenum and entire small intestine
4	25,000	14 hr.	Stomach and duodenum	Duodenum and entire small intestine
1	100,000	14 hr.	Stomach and duodenum	Duodenum and entire small intestine
2	400,000	14 hr.	Stomach and duodenum	Duodenum and entire small intestine
2	25,000	26 hr.	Traces in duodenum	Duodenum and entire small intestine
1	25,000	36 hr.	—	Duodenum and entire small intestine
1	25,000	2 days	—	Duodenum and entire small intestine
2	25,000	4 days	—	Lower part of duodenum and upper part of ileum in small amounts
3	25,000	6 days	—	Lower part of duodenum and upper part of ileum in small amounts
1	25,000	12 days	—	Smallest amount in the midportion of small intestine
2	25,000	14 days	—	—

Influence of the Interval of Time Between Feeding and Examination and of the Amount of Vitamin A Fed on the Vitamin A Absorption.—Vitamin A fluorescence in the intestinal wall was missed fifteen minutes but was found twenty-five minutes after administration of large doses of vitamin A (from 25,000 to 400,000 units) (table). In animals killed more than three hours after the feeding of vitamin A the site and the peak of absorption did not vary with the amount of vitamin A fed.

From three hours to four days after administration, vitamin A fluorescence was seen in the entire wall of the small intestine. After this time vitamin A fluorescence was encountered only in patchy distribution until it disappeared completely two weeks after administration of the large standard doses of vitamin A.

The amount of vitamin A in the intestinal wall increased from the beginning of the duodenum up to the border between the upper and middle thirds of the small intestine. From this point on it gradually decreased down to the ileocecal valve. The wall of the large intestine was free of vitamin A fluorescence in all the animals examined (fig. 1). In the lumen vitamin A was found in the stomach and the upper part of the duodenum within fifteen minutes, in the first part of the small intestine within twenty-five minutes and in the upper third of the small intestine one to three hours after feeding. Vitamin fluorescence was still noted in the content of the stomach and duodenum six to twenty-six hours after administration, while the content of the remaining intestinal tract was free of it. After twenty-six hours it had disappeared also from the stomach and duodenum.

Fat was demonstrable in the wall where vitamin A fluorescence was seen except in animals killed within one hour after feeding. In them no vitamin A fluorescence was seen, but fat was noted in the wall of the lower part of the small intestine. In the feces small amounts of fat were found throughout the entire length of the intestinal tract, including the large bowel.

Histologic Observations on the Absorption of Vitamin A in the Intestine.—If large amounts of vitamin A were given, fluorescence was imparted by almost the entire cytoplasm of the epithelial cells lining the villi of the small intestine. The striated border, however, was free of vitamin A; the droplets containing vitamin A were largest at the base of the epithelial cell. In the upper and lower parts of the small intestine only the epithelial cells of the upper half of the villi contained vitamin A, while at the border between the upper and the middle third of the intestine, the peak of the absorption, vitamin A fluorescence was found in the entire length of the villi. In the lamina propria of the villi the vitamin A fluorescence was unevenly distributed and imparted by the cytoplasm of some cells of the character of histiocytes or leukocytes (fig. 2*A*). The nuclei revealed no fluorescence (fig. 2*B*).

In the upper and distal parts of the small intestine fatty material with little vitamin A fluorescence was seen in the lacteals, whereas around the border between the upper and middle thirds of the small intestine the lacteals of the entire wall were filled with fatty material showing strong vitamin A fluorescence (fig. 2*C*). In the villi the lacteals appeared as thin fluorescent shreds, whereas in the subserosa they were seen as interlacing wide channels filled with fluorescent material. Sections stained with phosphin 3R showed a similar distribution of the fat.

Influence of Various Drugs on the Absorption of Vitamin A.—When 15 mg. of alpha tocopherol was given with 25,000 units of vitamin A (3 rats), no influence on the absorption of vitamin A was noted. In 4 rats which had received 5 mg. of atropine sulfate per kilogram thirty minutes before and thirty minutes after the feeding of 25,000 units of vitamin A the intestinal wall revealed much less vitamin A than was observed in controls, whereas the intestinal content of almost the entire small intestine revealed vitamin A fluorescence (fig. 1). In 5 rats which received

0.2 mg. of neostigmine methylsulfate per kilogram thirty minutes before and thirty minutes after the feeding of 25,000 units of vitamin A the vitamin A fluorescence in the intestinal wall was increased, while it disappeared from the intestinal content at a higher level than in control animals (fig. 1).

Demonstration of Vitamin A in Human Feces.—The feces of 15 patients who were hospitalized for various diseases were studied. Previous to examination 8 patients had received 75,000 units and 7 had received 400,000 units of vitamin A. Only in 2 patients were very small amounts of quickly fading vitamin A fluorescence found. One of them (with acute hepatitis) received 75,000 units and the other (with chronic nephritis) 400,000 units of vitamin A, twenty-four hours before the bowel movement occurred. The remaining 13 patients, who suffered from varying diseases, such as chronic nephritis, bronchogenic carcinoma, organic heart disease and cirrhosis of the liver, showed no vitamin A fluorescence in the feces even when repeatedly examined.

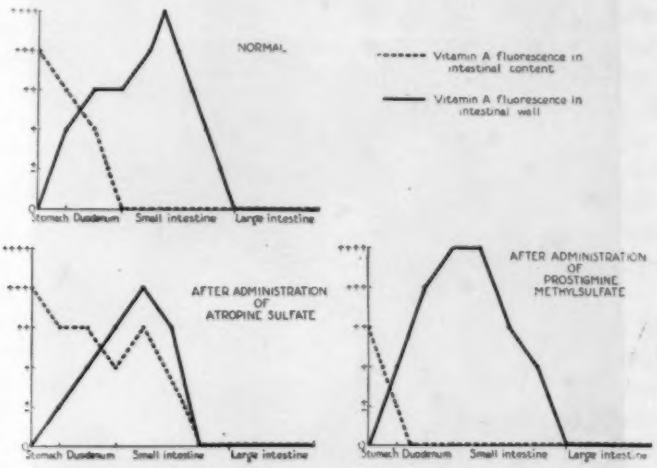

Fig. 1.—Curves of the estimated vitamin A fluorescence in the content and the wall of the gastrointestinal tract after oral administration of 25,000 units of vitamin A. A comparison is made between normal rats and two groups of rats which received 5 mg. of atropine sulfate per kilogram and 0.2 mg. of neostigmine methylsulfate per kilogram, respectively, thirty minutes before and thirty minutes after feeding. The animals were killed fourteen hours after the administration of vitamin A.

COMMENT

The fluorescent microscopic investigation of the intestine showed that the absorption of vitamin A given in oily solution runs parallel to that of fat. It is absorbed like fat in the entire small intestine, the peak being at the border between the upper and middle thirds of the small intestine. The passage of vitamin A through the intestinal wall resembles that of neutral fat also in the histologic picture.

No neutral fat or vitamin A is demonstrable by morphologic methods at the poles of the epithelial cells below the striated border. The absence of fat is probably due to saponification of the neutral fat, which is apparently reesterified in the middle part of the epithelial cells. Vitamin A fed in the esterized form is split in the intestine to vitamin A alcohol,[8a] which has a weaker fluorescence than vitamin A ester.[8b] This phenomenon may account for the reduction or absence of fluorescence in the lumen of the small intestine or at the internal poles of the epithelial cells, whereas in the rest of the intestinal wall vitamin A is again esterified and more strongly fluorescent.

The route of the vitamin A to the lymphatics within the villi is problematic like that of fat. The morphologic picture suggests that vitamin A is carried by mesenchymal cells, leukocytes or histiocytes, to the lymphatics. In those cells it may be stored for a considerable time. That explains why the absorption into the lacteals may be protracted for fourteen days.

In the intestinal content fat and vitamin A act differently. Whereas fat is found in the content of the entire intestinal tract, its fluorescence disappears in the proximal part of the small intestine even if extremely large doses of vitamin A are given. Likewise, in the human subject no vitamin A fluorescence was found in the feces after the administration of large amounts of vitamin A except in 2 patients, one of whom had acute hepatitis and the other chronic nephritis. The fact that Wald, Carroll and Sciarra after feeding large doses of vitamin A to normal persons found very small amounts of this vitamin in the feces and the fact that others found similar amounts in the feces of

11. Footnote deleted.

rats are not in disagreement with our findings, because our method, while less sensitive, is surely indicative of the trend.

Two explanations for the disappearance of the vitamin A fluorescence of the fat in the upper part of the small intestine present themselves: 1. Almost the entire vitamin A present is absorbed in the proximal part of the intestine, reducing the concentration of the vitamin in the content to a level beyond demonstration by the fluorescence method. If that is so, extensive destruction of the absorbed vitamin A within the organism has to be assumed in view of the known noneconomic utilization of vitamin A. 2. It has to be considered that large amounts of vitamin A are destroyed within the abdominal

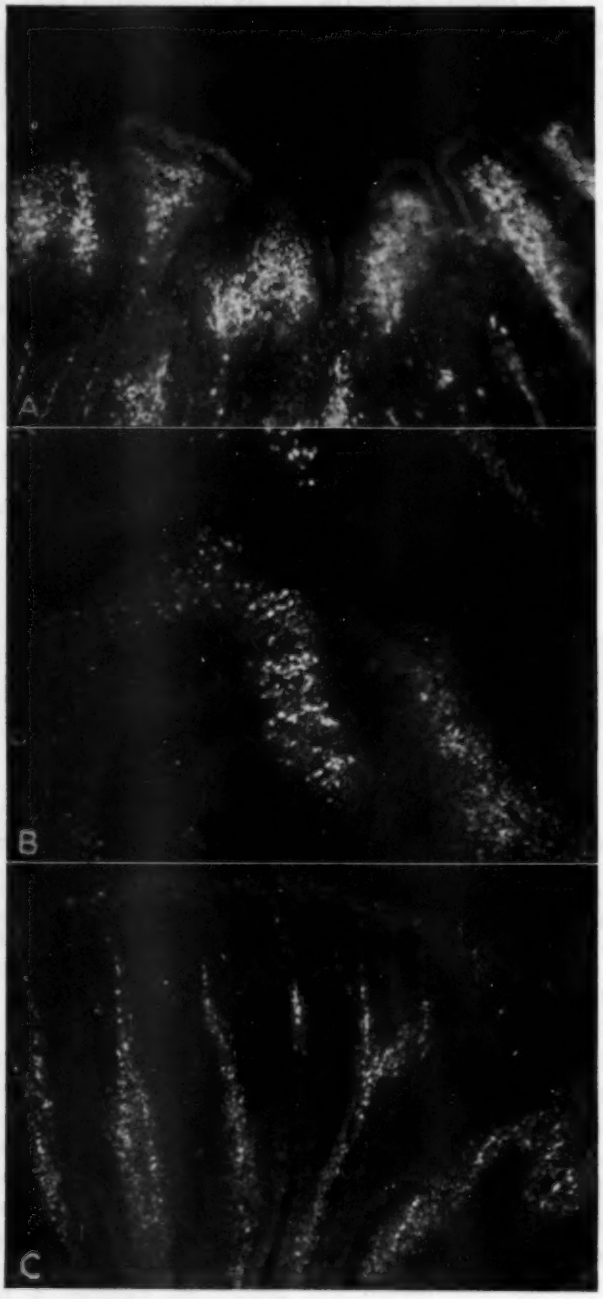

Fig. 2.—*A*, vitamin A fluorescence of the villi of the small intestine. In the lamina propria the vitamin A fluorescence is mainly imparted by cells of histiocytic or leukocytic character. *B*, vitamin A fluorescence in the epithelial cells, the lamina propria and the lacteals of the villi of the small intestine. The dark spots in the epithelial cells indicate the nuclei, which are free of vitamin A fluorescence. *C*, vitamin A fluorescence in cells of the villi and in the lacteals of the villi and of the subserosa of the small intestine.

content. The intestinal bacteria do not destroy vitamin A[12] and can therefore not be made responsible for this destruction. Vitamin E or tocopherol has been repeatedly claimed to be an antioxidant which protects vitamin A in the organism.[13] In our experiments a protective effect in the intestine was not apparent.

The fact that vitamin A persists longer in the stomach and the duodenum than in the small intestine agrees with both mentioned hypotheses. According to our findings, the noneconomic utilization of vitamin A is not caused by insufficient absorption in the intestinal tract and by loss through the feces but by destruction of the vitamin either within the organism or more probably within the intestinal lumen. Apparently not a defect of the absorptive ability of the intestine but an increase in destruction seems to be a factor in endogenous vitamin A deficiency.

The presented observations agree best with the hypothesis that vitamin A and tocopherol which originally are fed together become separated when the fat, which carries tocopherol and vitamin A, is saponified. Vitamin A separated from fat and the protective antioxidant and forming a fine film[14] is probably more vulnerable to destruction until it is taken up by the reesterified fat within the villi of the intestine. The protective action of the tocopherol in the tissues is established.[15] The destruction of vitamin A or carotene by oxidation, especially by unsaturated fatty acids or other substances occurring in the intestine, has been described.[16]

The administration of drugs was shown to have marked influence on the absorption of vitamin A. Atropine, which interferes with the absorption of vitamin A in man,[17] reduced absorption in the intestinal wall and increased the vitamin A fluorescence in the intestinal content. Neostigmine revealed its antagonistic effect to atropine by increasing vitamin A in the wall and reducing it in the intestinal content. The mechanism involved appears to be an influence on the intestinal motility, since the absorption-promoting neostigmine influences primarily the peristalsis.

The visualization of the intestinal absorption of vitamin A appears to be a simple method to study the effect of drugs on the small intestine as far as the absorption of fat-soluble vitamins is concerned.

SUMMARY

By fluorescence microscopic examination of the rat intestine it was shown that vitamin A in oily solution is absorbed similarly to fat in the entire small intestine, the peak being at the border between the upper and middle thirds. The absorption takes place by the lymphatic route. Vitamin A is apparently carried by neutral fat from the epithelial cells to the lacteals.

The absorption starts within twenty-five minutes after feeding if large doses of vitamin A are administered and continues for a long time because of storage of vitamin A in the mesenchymal cells of the villi.

Vitamin A disappears from the lumen in the proximal part of the small intestine, which is explained either by almost complete absorption with consecutive destruction in the tissue or more probably by extensive destruction of vitamin A in the lumen while it is separated from the saponified fat and the protecting antioxi-

neostigmine promotes the absorption of vitamin A is therefore not explained by a limited ability of the intestinal wall to absorb it and consecutive loss with the feces.

No influence of tocopherol on the absorption of vitamin A was observed. Atropine retards and neostigmine promotes the absorption of vitamin A. The fluorescence microscopic examination of the intestinal wall and content for vitamin A in rats is suggested as a suitable method for the pharmacologic study of intestinal absorption.

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FORMATION OF HEMOSIDERIN IN THE LUNGS

AN EXPERIMENTAL STUDY

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The presence of hemosiderin-containing macrophages in the pulmonary alveoli of persons whose circulation was presumably normal until a short time before death suggests that in certain circumstances this pigment frequently forms within forty-eight and occasionally within twenty-four hours after free erythrocytes have gained access to the air sacs. The following experiments were undertaken to determine the manner and the rate at which the lungs of rabbits dispose of free intra-alveolar blood and the circumstances in which hemosiderin is formed.

EXPERIMENTAL PROCEDURE

Blood was withdrawn from the aural vein of a rabbit in a heparin-containing syringe. The animal was then anesthetized with pentobarbital sodium and the withdrawn blood injected into the lumen of the trachea through a no. 26 gage needle. Because of coughing, not more than approximately 0.5 cc. (blood, blood–dextrose solution mixture or dextrose solution) could be injected at one time. A total of between 3 and 6 cc. was injected in 0.5 cc. aliquots over a period of between five and ten minutes. Even though all possible precautions were observed, it was still found that some of the aspirated material was lost by reflex coughing. Thus, it was found that a total of approximately 3 cc. of whole blood or 6 cc. of whole blood–dextrose solution mixture must be injected in order to insure that more than 2 cc. of blood would be trapped in the lungs.

Four groups of animals were used. In the first group 3 to 4 cc. of whole blood was injected. In the second, the injection consisted of a mixture of whole blood and hypertonic solution of dextrose (20 to 25 per cent). Approximately 3 cc. of whole blood mixed with an equal amount of the dextrose solution was used. In the third, the hypertonic solution of dextrose alone was injected. In the fourth, whole blood alone was injected immediately after death. A total of 32 rabbits was used.

After intervals varying from three hours to two weeks the rabbits were killed by injection of pentobarbital sodium.

Several animals received a second injection of whole blood, a mixture of blood and dextrose solution or dextrose solution alone after an interval of one to two weeks and were killed three hours after the second injection. In all animals the trachea was ligated immediately after death, and the organs of the chest and the neck were removed in toto and after gross inspection were fixed in neutral 4 per cent solution of formaldehyde. Multiple sections of the lungs were taken and stained with hematoxylin-eosin and by Gömöri's modification of the hemosiderin stain.

From the Department of Legal Medicine, Harvard Medical School, the Office of the State Pathologist, Massachusetts Department of Mental Health, and the Laboratory of the Metropoljtan State Hospital.

RESULTS

GROUP 1. — These rabbits were given intratracheal injections of whole blood alone.

First Twenty-Four Hours.—Four to ten hours after death, disseminated dark blue areas of trapped blood were observed throughout both lungs. These foci were most extensive in the right lower pulmonary lobe, presumably because of the more vertical course of its major bronchus. At the peripheral portions of the lungs there was slight emphysema. The lungs generally showed moderate hyperemia and edema.

Microscopically, the smaller bronchi and many groups of alveoli were filled with aspirated red cells. There were scattered emphysematous and atelectatic areas. Where the blood had collected, there was infiltration by leukocytes, including many eosinophils. Within four hours there were definite hypertrophy and proliferation of the septal cells (fig. 1). Even in this early stage many of the septal lining cells had desquamated and migrated into the lumens of the air sacs. At ten hours the septal proliferation was still more pronounced and the number of free macrophages increased. Some of them had erythrocytes engulfed within them. In some instances the phagocytes containing red cells were connected with the alveolar walls, whereas in other instances the macrophages were lying free in the lumen. Stains for hemosiderin at this period showed none present.

After Twenty-Four Hours.—Grossly the red discoloration of the lungs had disappeared. Here and there were small areas of brown discoloration. On microscopic examination only occasional free erythrocytes were encountered. Likewise the cellular reactive phase had subsided. Proliferation of alveolar lining cells was still definite. Some of the focal emphysematous and atelectatic areas still persisted. Macrophages containing red cells were not seen. Stains for hemosiderin showed that it was entirely absent.

GROUP 2. — These rabbits were given intratracheal injections of a mixture of whole blood and dextrose solution.

First Twenty-Four Hours.—In the first twenty-four hours the gross evidence of aspiration of blood was similar to that encountered in the rabbits of group 1, which received blood alone. Well defined areas of trapped blood were seen in all lobes, those in the right lower pulmonary lobe being usually most marked and those in the hilar regions being particularly prominent. One important distinction from the animals given whole

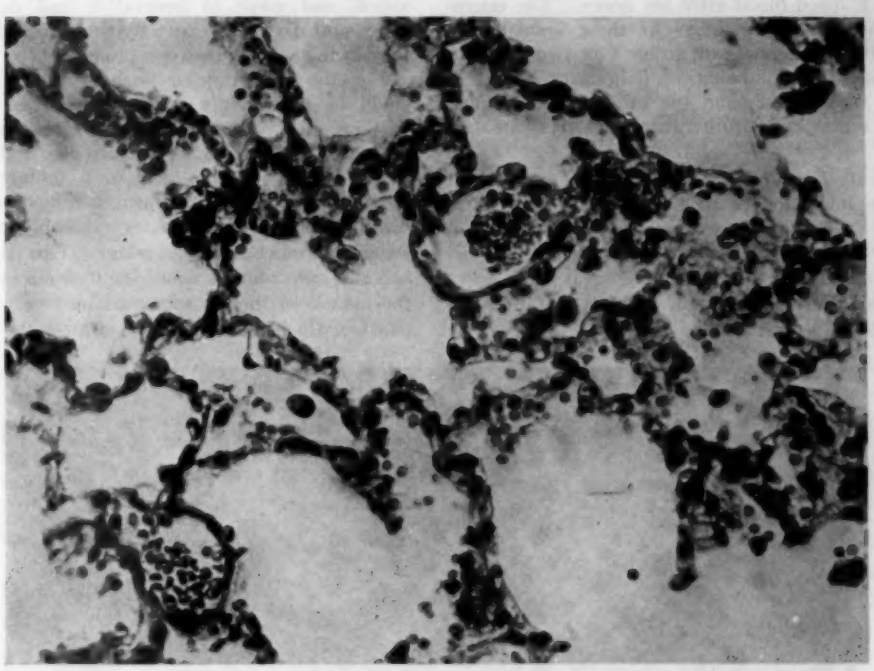

Fig. 1.—Lung of a rabbit four hours after intratracheal injection of approximately 4 cc. of its own blood. Note the proliferation and desquamation of alveolar lining cells. Hematoxylin and eosin stain; × 420.

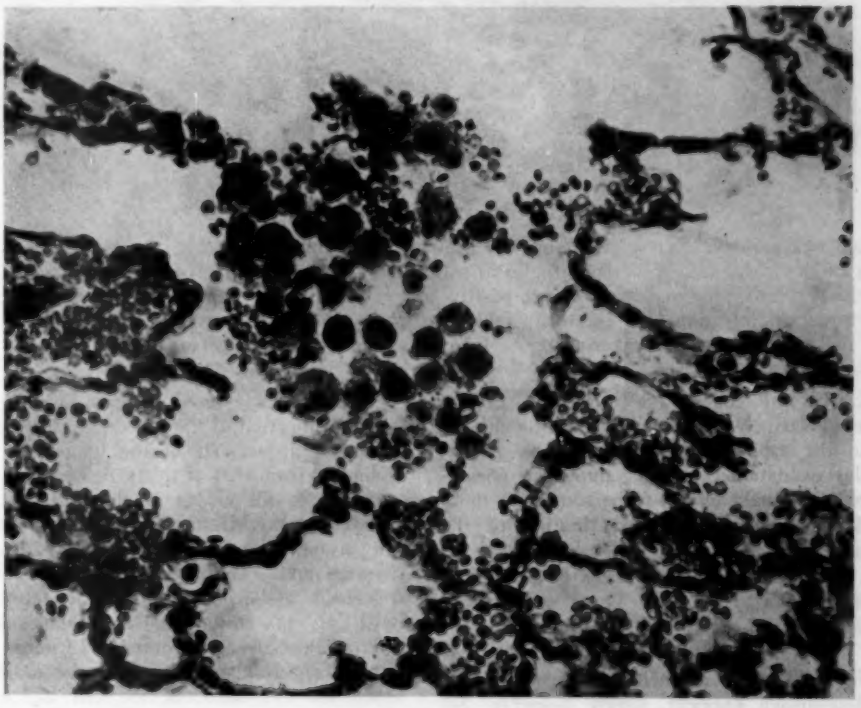

Fig. 2.—Lung of a rabbit ten hours after intratracheal injection of approximately 3 cc. of its own blood mixed with an equal volume of a hypertonic solution of dextrose. The large number of detached phagocytic alveolar lining cells suggests that there has been interference with their migration out of the lungs. Hematoxylin and eosin stain; × 420.

blood without dextrose solution was the much greater degree of edema and hyperemia and the large size of the areas of trapped blood after ten hours. The microscopic appearance of the lungs of these animals differed from that of animals of group 1 in two respects. One was the greater amount of pulmonary edema in the animals of group 2, and the other was the more pronounced septal cell proliferation and desquamation (fig. 2).

After Twenty-Four Hours.—Twenty-four hours after injection, the lungs looked pale and emphysematous at their borders, and there was extensive brown discoloration near the hilus, with smaller brown areas distributed throughout all lobes. These areas were more extensive than those observed in the lungs of the rabbits comprising the first group.

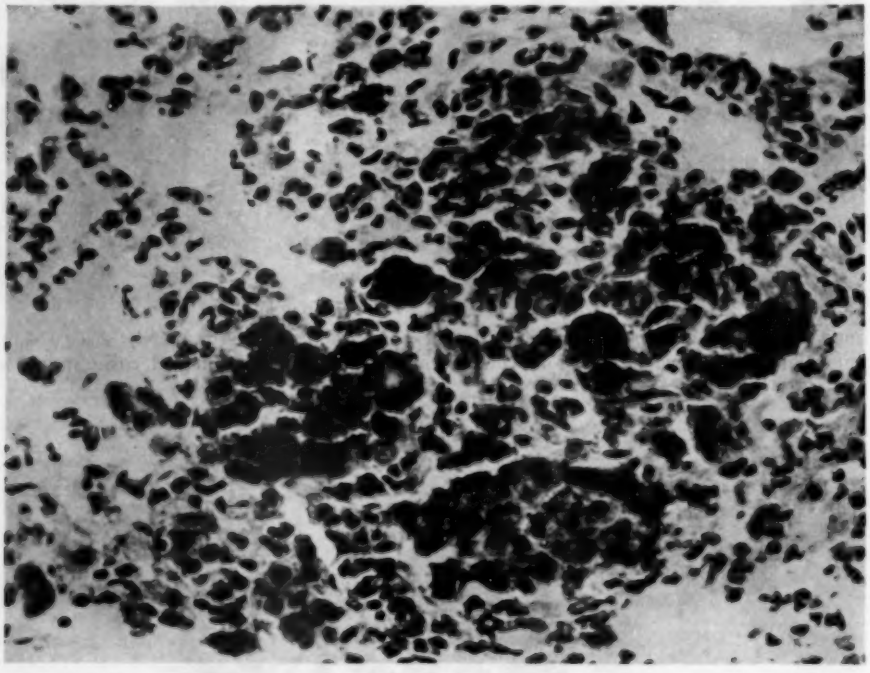

Fig 3.—Lung of a rabbit forty-eight hours after intratracheal injection of its own blood mixed with a hypertonic solution of dextrose. Many of both the attached and the desquamated alveolar lining cells give a strongly positive reaction for hemosiderin. The earliest positive reaction for hemosiderin was observed twenty-four hours after the intratracheal injection of a mixture of blood and dextrose solution and consisted of a faint blue discoloration of the cytoplasm of the macrophages. Gömöri's stain for hemosiderin; × 420.

Microscopically, red cells were numerous in the alveoli but were relatively infrequent in the bronchioles. In relation to the aspirated blood there was a pronounced cellular exudation containing numerous eosinophils. This reactive phase tended to be somewhat more widespread than the concomitant distribution of the aspirated blood cells.

The edema was still severe, and areas of atelectasis and emphysema were also present. There was marked septal cell proliferation, and numerous macrophages were present within the alveoli. Some of the macrophages contained red cells in their cytoplasm; others contained yellow-brown pigment, which, however, did not give a positive reaction for hemosiderin. Presumably this pigment represented a precursor of hemosiderin.

Thirty-three hours after the injection, small brown areas were visible in all lobes, but otherwise the lungs were not outstandingly abnormal macroscopically. Microscopically, a moderate degree of edema still persisted, and while in general it had decreased, there were still foci of relatively dense cellular infiltration in which eosinophils were prominent.

For the first time a positive hemosiderin reaction could be consistently obtained[1] and was observed in the free macrophages in the alveoli as well as in the cells still connected with the alveolar walls. The reaction usually took the form of a uniformly faint blue discoloration of the cytoplasm, but in others there were definite small blue granules, probably representing a somewhat more advanced stage. One striking feature was the pronounced septal cell proliferation, although the lumens of the air sacs contained only a few macrophages, which had become separated.

At about forty-seven to fifty hours after the intratracheal injection of the blood–dextrose solution mixture the gross appearance of the lungs was not strikingly dissimilar from that at thirty-three hours. Small brown areas were still visible in the lobes, especially in the hilar regions. Microscopically, many of the red cells had disappeared from the alveolar lumens, and emphysema and edema were still visible. Focal areas of atelectasis associated with minor cellular infiltration, consisting of eosinophils and hemosiderin-containing macrophages, were observed. In most instances, the hemosiderin was granular, and the earlier reaction, in which the cytoplasm of the cell had stained a light uniform blue, had in large part disappeared. The proliferation of septal cells was still prominent. Most of the macrophages containing hemosiderin were con-

1. A positive hemosiderin reaction was found in 1 rabbit after twenty-four hours.

nected with the septal wall, and only a few of them were found lying free in the alveolar lumen. An interesting observation was that the number of free macrophages had diminished (fig. 3).

After seven days the lungs appeared grossly normal. Microscopically, the red cells had disappeared completely from the air sacs. Although a few small atelectatic areas persisted, the concomitant inflammatory reaction accompanying it was of less intensity than that in animals killed at earlier periods. Septal proliferation, chiefly in relation to the atelectatic foci, was still visible, and a significant proportion of the cells contained dark bluish granules of hemosiderin. Such macrophages were ordinarily observed in the alveolar walls about the bronchi but rarely in the alveolar lumens or in the peribronchiolar lymphoid tissue. Free intra-alveolar macrophages containing hemosiderin were rarely found after seven days.

In 3 rabbits a second injection was made subsequent to the first administration of the mixture of blood and dextrose solution. In 2 of them the second injection consisted of a mixture of blood and hypertonic dextrose solution seven and fourteen days, respectively, after the first injection. In the third rabbit the second injection consisted of the animal's whole blood without dextrose, and the injection was made seven days after the first injection. All 3 animals were killed three hours after the second injection. The gross picture was similar in all 3 rabbits. All lobes of both lungs showed dark bluish areas of trapped blood originating from the second injection. No brownish areas could be seen grossly. Microscopically, there were marked edema and hyperemia, focal areas of atelectasis and compensatory emphysema. There was intense proliferation of septal cells, and many of the so-called "resting macrophages," which presumably had previously been attached to the septal wall, were now lying free in the lumens of the alveoli, which contained the aspirated red cells from the last injection. Many of the macrophages contained dark blue-stained hemosiderin granules. It was apparent that the second injection had provided a stimulus for the separation of the cells from the septal walls and their consequent migration into the alveolar lumens (fig. 4). An incidental observation in 1 rabbit was a circumscribed focus of granulation tissue in the parenchyma of the lungs in which hemosiderin-containing phagocytes were found. Similar macrophages were observed in the peribronchiolar lymphoid tissue of this animal. A similar mobilization of macrophages with and without hemosiderin was obtained if a hypertonic solution of dextrose was used for the second injection instead of blood, though the reaction was not so extensive as in the other 3 rabbits, which had received blood for the second injection.

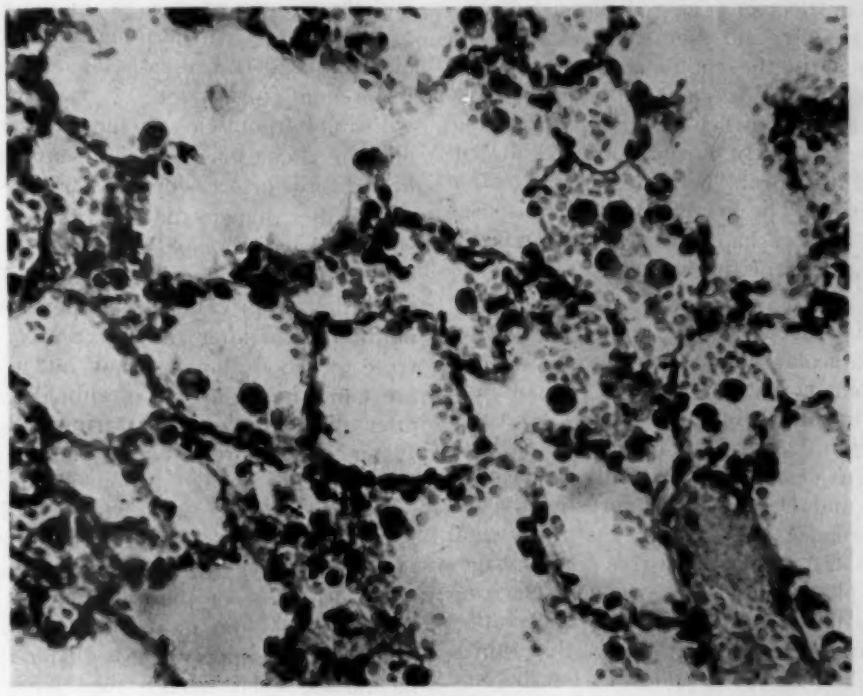

Fig. 4.—Lung of a rabbit three hours after the second injection of its own blood. The first injection occurred seven days previously and consisted of blood and dextrose solution. Many alveolar lining cells, some of which contained hemosiderin derived from the previous injection of blood, have been activated and now lie free in the alveoli. Gömöri's stain for hemosiderin; × 420.

GROUP 3.—These rabbits were given intratracheal injections of a hypertonic solution of dextrose.

When 20 to 25 per cent dextrose solution was introduced, pronounced edema and emphysema of the lungs were observed. After six hours some septal proliferation and a few eosinophils and macrophages were present in the alveolar lumens together with a few red cells. However, the effect of the dextrose solution was transitory and had disappeared in animals after twenty-four hours.

GROUP 4.—These rabbits were given intratracheal injections of whole blood or of a mixture of whole blood and dextrose solution immediately after death.

Two rabbits which had not received previous injections were given intratracheal injections of whole

blood alone and whole blood–dextrose mixture, respectively, immediately after death from pentobarbital sodium anesthesia. At autopsy, after the injection, large red-purple areas of the injected blood were visible in all lobes of the lungs in both animals, particularly in the lower lobes of the right lungs. Microscopically, blood was visible in many of the small bronchi and in many of the alveoli. However, there was no significant postmortem reaction to the presence of the injected material with the exception that a few free macrophages were seen in the alveolar lumens.

COMMENT

Within four hours after homologous blood enters into the alveoli of the rabbit's lung, hypertrophy, hyperplasia and desquamation of the alveolar lining cells occur. These reactive changes reach their fastigium within about ten hours and show marked recession by the end of twenty-four hours. The red blood cells are disposed of rapidly, and even though an amount of blood equal to the combined weight of the lungs enters the air passages, little or no trace of it will be found when the animal is killed twenty-fours later. In normal animals the injected blood disappears without local formation of hemosiderin. The erythrocytes are disposed of in part and perhaps entirely through the phagocytic activity of the activated alveolar lining cells.

Proliferation of septal cells and macrophages free in the alveolar lumens have been observed under different conditions in cases of acute and chronic inflammation of the lungs and after aspiration of foreign material.[2] These reactions have recently been described in detail by Geever, Neubuerger and Davis,[3] who expressed the belief, in accordance with others, that the septal cells provide the majority of free macrophages or alveolar phagocytes. They concluded from their observations that some of the proliferating septal cells form the alveolar lining, that others remain attached to the septal wall but become phagocytes, while still others may become detached to form free phagocytes. It may be inferred that the proliferation of septal cells is a nonspecific reaction incident to the presence of an irritating agent in the alveoli. My observations confirm the opinion of Geever, Neubuerger and Davis so far as the reaction following the aspiration of blood is concerned.

As early as 1877, H. Nothnagel found that blood injected into the trachea of the rabbit disappeared rapidly from the pulmonary alveoli.[4] As a rule, an aspirated fluid disappears quickly from the alveoli, but resorption may be delayed if the lungs are concomitantly rendered edematous and hyperemic by the intratracheal administration of a hypertonic solution of dextrose.[5] My observations confirm this statement with relation to the injection of blood into the trachea. Injection of a mixture of blood and 20 to 25 per cent dextrose solution makes it possible to study the reactions following the aspiration of red cells into the air sacs.

If the normal absorptive properties of the respiratory membranes of the rabbit are disturbed as occurs temporarily following the entrance of a hypertonic solution of dextrose into the alveoli, there are differences in both the rate and the manner of disposal of the intra-alveolar erythrocytes. A hypertonic solution of dextrose alone causes transitory pulmonary edema and hyperemia (for as long as twenty-four hours) with mild stimulation of the alveolar lining cells and the elicitation of a slight exudative reaction in the form of occasional eosinophils.

When a hypertonic solution of dextrose is combined with homologous blood, both the activation of the alveolar lining cells and the elicitation of an exudative response is more pronounced than after the injection of either dextrose or blood alone. Many intact erythrocytes are found in the alveoli of animals killed on and after the second day. During the second day (after thirty-three hours) large amounts of hemosiderin can be demonstrated in both the desquamated and the attached hypertrophic alveolar cells. By the end of the fourth day most of the intact erythrocytes have disappeared, as have also most of the wandering macrophages. Although the desquamated phagocytic alveolar lining cells disappear, those that retain their attachments, even though they have engaged in phagocytic activity and contain hemosiderin, may retrogress in size, remain in situ and are capable of being reactivated by subsequent stimulation. Although the maximum period of survival of these attached hemosiderin-containing alveolar lining cells was not determined, it was noted that they were susceptible to reactivation as long as fourteen days after an episode of phagocytic activity. When two weeks after the first intratracheal injection of blood the procedure was repeated it was observed that within four hours these hemosiderin-containing cells underwent hypertrophy and desquamated into the alveoli, where they participated in the phago-

2. Foot, N. C.: Am. J. Path. **3**:413, 1927. Gardener, L., and Smith, D.: ibid. **3**:445, 1927. Robertson, O.: Physiol. Rev. **21**:112, 1941. Fried, M. B.: Arch. Path. **12**:689, 1931. Clement, L. P.: ibid. **17**:76 1934. Wright, A.: Am. J. Path. **11**:497, 1935. Ross, J.: Arch. Path. **27**:478, 1939.

3. Geever, E. F.; Neubuerger, K. T., and Davis, C. L.: Am. J. Path. **19**:913, 1943.

4. Nothnagel, H.: Virchows Arch. f. path. Anat. **71**:415, 1877.

5. Gordonoff, T.: Ergebn. d. Physiol. **40**:53, 1938.

cytosis of the newly arrived erythrocytes. It was inferred, therefore, that the hypertrophy and the phagocytic activity of alveolar lining cells represent a reversible change so long as the cells do not desquamate.

Hemosiderin was first observed after twenty-four hours and took the form of a faint blue and more or less uniform discoloration in the cytoplasm of the macrophage. Some of these macrophages were free in the alveolar lumen, whereas others were attached to the septal wall. Thirty-one hours after the injection of the blood-dextrose mixture, more macrophages were found containing hemosiderin. After forty-eight hours a positive hemosiderin reaction was obtained in all experiments. The appearance of the hemosiderin gradually altered. At first there was a diffuse distribution of the substance throughout the cytoplasm of the macrophage, and with stains for hemosiderin a faint blue discoloration developed, which in some instances was almost imperceptible. Subsequently it assumed a granular character, and at the end of a week it was visualized as small dark compact blue-staining masses.

SUMMARY

In normal circumstances most or all of the erythrocytes injected into the alveoli of a rabbit's lung disappear during the first twenty-four hours.

The disappearance of intra-alveolar erythrocytes is due in part or perhaps entirely to the activity of alveolar lining cells and in normal circumstances does not lead to the formation of hemosiderin in the lungs.

In the presence of a transitory pulmonary edema caused by combining the erythrocytes with a hypertonic solution of dextrose, their disappearance from the lungs may be delayed for as long as a week, and hemosiderin appears in the alveolar macrophages during the second day.

Hypertrophy and phagocytic activity of alveolar lining cells do not constitute an irreversible change so long as the cells retain their septal attachments. Attached alveolar lining cells containing hemosiderin may be reactivated and mobilized as long as two weeks after their original participation in the disposal of intra-alveolar blood.

SECONDARY CARCINOMA OF THE ESOPHAGUS AS A CAUSE OF DYSPHAGIA

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In March 1942 Gross and Freedman [1] reported a case of esophageal obstruction produced by a metastasis from a carcinoma of the prostate. Their report included a survey of the literature. They were able to find recorded 42 cases of secondary tumor of the esophagus, but in no instance had an esophageal metastasis produced symptoms. They concluded that metastatic tumor involving the esophagus is exceedingly rare and remarked that their case was the first recorded instance of an obstructing secondary tumor of the esophagus. Shortly after the appearance of their paper there came to autopsy in the department of pathology at the Pathological Institute of McGill University 3 patients who had suffered symptoms of esophageal obstruction which was proved by postmortem examination to have been caused by secondary involvement of the esophagus by cancer. This coincidence in the light of the statement of Gross and Freedman that the occurrence of this condition is rare stimulated a search of the autopsy records of this institute for similar cases, and this paper is a brief report of the findings.

A total of 3,700 consecutive autopsies was first reviewed to determine the incidence of cancer in general and of secondary involvement of the esophagus in particular. Exclusion of the instances of stillbirth and neonatal and infant death left a general autopsy population of 3,048, among which 599 cases of cancer were encountered (19.7 per cent). This figure excludes all cases of primary intracranial tumor except rare instances of cancer of intracranial origin capable of metastasizing elsewhere. Fifteen instances of multiple cancer (2.5 per cent of all cases of cancer) were counted as single cases, although in several there were metastases from both primary tumors. Secondary involvement of the esophagus occurred in 19 of the 599 cases of cancer, an incidence of 3.2 per cent. Seven additional cases of secondary tumor of the esophagus were found in the autopsy records apart from the 19 cases encountered among the 3,700 consecutive autopsies initially reviewed. Thus a total of 26 cases of secondary involvement of the esophagus by tumor were available for analysis.

Of the 26 metastasizing tumors, 24 were carcinomas, 1 was a lymphosarcoma and 1 a cancer of undetermined origin and type, probably a melanoma. The organs in which the tumors originated and the numbers for each were as follows: trachea or bronchus, 8; stomach, 7; larynx, 4; breast, 2; pancreas, 2; testis, 1; mediastinal lymph nodes, 1; origin undetermined, 1.[2] In the cases of primary carcinoma originating in the larynx, the trachea, the bronchi or the stomach the involvement of the esophagus was usually by direct extension, although in some cases definite metastases existed as well. However, involvement by direct invasion could not be excluded except in 1 instance in which a primary carcinoma of the stomach had originated in the region of the pylorus and had not invaded the remainder of the stomach. If the latter case is included, there were 8 instances (about 30 per cent of all cases of secondary tumor of the esophagus) in which the involvement of the esophagus was definitely metastatic from a more or less distant primary tumor. If the clearly metastatic esophageal tumors encountered in this study are grouped together with the similar ones collected by Gross and Freedman,[1] the total is 23 esophageal tumors metastatic from primary tumors located as follows: stomach, 7; breast, 3; larynx, pancreas and testis, 2 each; eye, tongue, bronchus, mediastinal lymph nodes, prostate, tibia and an undetermined site, 1 each. The variety of the organs included in this list appears to indicate that cancer of any organ probably can metastasize to the esophagus.

From the Department of Pathology, Pathological Institute, McGill University, Montreal, Canada.

1. Gross, P., and Freedman, L. J.: Arch. Path. **33**:361, 1942.

2. The single case of primary carcinoma of the trachea was included with an excellent illustration in a paper on this subject by Culp (J. Thoracic Surg. **7**:471, 1938), while 1 of the 2 cases of esophageal metastasis from primary carcinoma of the pancreas was mentioned by Grauer in a paper on pancreatic carcinoma (Arch. Int. Med. **63**:884, 1939).

Secondary carcinoma of the esophagus, including that which invades by direct extension, is often accompanied by symptoms of esophageal obstruction. In the present study of 26 cases, partial or complete obstruction of the esophagus with corresponding symptoms was encountered in 12 cases, nearly half of the total. In 2 cases a primary tumor of the larynx had extended into the upper portion of the esophagus, constricting that region; in 2 cases there was extension of a primary carcinoma of the stomach into the lower end of the esophagus, constricting that portion; in 5 cases a primary carcinoma of the bronchi or of the lower end of the trachea caused stenosis of the midportion of the esophagus by direct invasion. In 3 of these cases the dysphagia was so severe that gastrostomy was required.

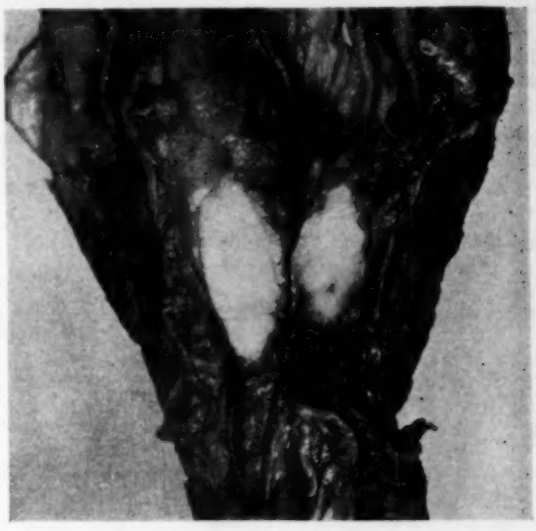
In the remaining 3 cases of esophageal obstruction caused by a secondary tumor of the esophagus, the involvement of the esophagus was by metastasis from a distant primary growth.

The first of the patients was a 53 year old woman who had undergone mastectomy three years earlier for carcinoma of the breast. About six months before death she began to experience dysphagia and regurgitation. When she finally returned to the hospital, she had lost 90 pounds (40.8 Kg.) in weight and had been unable to swallow either soft foods or liquids for twelve days. She was treated by gastrostomy but died two weeks later. At autopsy a metastatic tumor nodule was found in the wall of the esophagus opposite the bifurcation of the trachea, producing obstruction at that point.

The second patient was a 67 year old woman who had undergone mastectomy for carcinoma of the breast twelve years before her death. For eleven years after that she was well, but seventeen months before death she returned to the hospital complaining of dysphagia and loss of weight. Roentgen examination revealed stenosis of the esophagus. Five months later the narrowing could not be demonstrated, and the dysphagia was thought, therefore, to be of functional origin. Dysphagia, loss of weight and weakness increased progressively during the ensuing year until death supervened, at which time the patient's weight was only 60 pounds (27 Kg.). At autopsy the esophagus was found to be almost completely occluded at the junction of the middle and lower thirds by metastatic tumor nodules which occupied the wall of the esophagus without invading or interrupting the epithelial lining (figure).

The third patient was a 36 year old man who first noticed a small painless swelling of the right testis seven months before he was admitted to the hospital complaining of dysphagia and fatigability of two months' duration. Unilateral orchidectomy was performed, and the tumor nodule proved to be a histoid teratoma with an associated embryonal carcinoma of the testis; no definite chorioncarcinoma was found, although the urine showed a high content of estrogenic substance. The patient continued to suffer from dysphagia and later from dyspnea, retrosternal pain and persistent cough with bloody sputum. He died seven weeks after admission to the hospital. Autopsy revealed stenosis of the midportion of the esophagus due to occupation of its wall by hemorrhagic metastatic tumor nodules. Masses of hemorrhagic tumor tissue were also found in the mediastinum and the lungs. Microscopically, the tumor metastases had the characteristics of choriocarcinoma.

Three of the cases of dysphagia due to involvement of the midportion of the esophagus by direct extension of a primary carcinoma of the bronchus form an interesting group. In each instance the patient was a man in the late fifties, and each was under treatment for syphilitic aortitis with diffuse dilatation of the thoracic aorta. In 2 cases the coexistence of carcinoma of the bronchus was suspected and a bronchoscopic examination was undertaken. In one of these cases no tumor was found on biopsy from a constricted area in the lower end of the trachea;



Photograph of the esophagus, opened longitudinally, in the second case described in the text. A pale gray mass of tumor tissue, metastatic from a primary carcinoma of the breast, occupies the wall of the esophagus in a short segment and has produced extreme narrowing of the lumen. Before being opened, the latter barely admitted a small probe. The lining epithelial layer was grossly and histologically intact.

in the other case the tumor was seen, and biopsy proved it to be carcinoma. In the third case the presence of the tumor was disclosed only at autopsy. In each instance the symptom of moderately severe dysphagia had been interpreted as the result of compression of the esophagus by the expanding aorta. Meakins [3] remarked on the frequency of dysphagia in cases of saccular or fusiform aneurysm of the aortic arch; Cecil [4]

3. Meakins, J. C.: Practice of Medicine, ed. 3, St. Louis, C. V. Mosby Company, 1940, p. 428.

4. Cecil, R. L.: Textbook of Medicine, ed. 5, Philadelphia, W. B. Saunders Company, 1940, p. 1244.

made a similar reference. Price [5] stated that slight dysphagia is a common symptom of such aneurysms but is rarely an important one, although aneurysms may occasionally cause esophageal ulceration and may eventually rupture into the esophagus. The 3 cases just cited suggest that when moderately severe dysphagia accompanies diffuse dilatation of the aorta, it should be borne in mind that the dysphagia may have another cause, and investigations should be undertaken to rule out the possibility of obstruction of the esophagus by secondary tumor.

SUMMARY

Twenty-six cases of secondary involvement of the esophagus by cancer are analyzed. Of these, 19 cases were encountered among 599 consecutive cases of cancer in which autopsy was performed, an incidence of 3.2 per cent.

5. Price, F. W.: A Textbook of the Practice of Medicine, ed. 6, London, Oxford University Press, 1941, p. 1047.

Of the 26 cancers, 24 were carcinomas, 1 was a lymphosarcoma and 1 was a cancer of undetermined origin and type, probably a melanoma. The organs in which the tumors originated and the number for each were as follows: trachea or bronchus, 8; stomach, 7; larynx, 4; breast, 2; pancreas, 2; testis, 1; mediastinal lymph nodes, 1; origin undetermined, 1. There were 8 instances (about 30 per cent of all cases of secondary cancer of the esophagus) in which the involvement of the esophagus was definitely metastatic from a more or less distant primary tumor.

Among the 26 cases, partial or complete obstruction of the esophagus with corresponding clinical symptoms was encountered in 12 cases. In 3 of these cases the involvement of the esophagus was by metastasis from a distant primary tumor.

Secondary carcinoma of the esophagus is not rare and in somewhat less than half of the cases leads to more or less severe dysphagia.

NEPHROTOXIC EFFECT OF POISONS ACTIVE ON CONVOLUTED TUBULES IN PRESENCE OF HYDRONEPHROSIS OF ONE KIDNEY

POISONS STUDIED: URANIUM NITRATE, MERCURY BICHLORIDE, RACEMIC TARTARIC ACID AND DIETHYLENE GLYCOL

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In the course of a previous investigation it was noted that hydronephrotic kidneys were resistant to the nephrotoxic effect of acute poisoning with uranium nitrate and mercury bichloride.[1] As that study was concerned with another problem, an investigation was undertaken in which the purpose was to expand those experiments and make similar experiments with other drugs.

Frola [2] first reported that in an animal in which one kidney had been made hydronephrotic and the other left unobstructed the hydronephrotic kidney was resistant to chronic poisoning with uranium nitrate and that this resistance was proportional to the duration of the hydronephrosis. Apparently this general phenomenon was known previously.[3] Frola's explanation that the resistance of the hydronephrotic kidney is the result of excessive activity of the other kidney due to reabsorption of products of degeneration from the hydronephrotic kidney is inadequate.

It is possible to protect experimental animals from the nephrotoxic effects of drugs in different ways: Sodium carbonate,[4] sodium bicarbonate [5] and sodium citrate [6] protect the kidneys from severe uranium nitrate injury, while hemoglobin,[7] testosterone [8] and plasmaphoresis [9] protect against mercuric destruction of tubules.

Sucrose produces severe hydropic degeneration in both the hydronephrotic and the unobstructed kidney of the experimental rabbit at intervals of obstruction up to one month.[10]

In the present investigation uranium nitrate, mercury bichloride, racemic tartaric acid and diethylene glycol were administered to comparable series of animals with left unilateral hydronephrosis.

EXPERIMENTAL PROCEDURE AND OBSERVATIONS

In 26 rabbits anesthesia was induced by intravenous administration of pentothal sodium, and the left ureter was doubly ligated and severed, sterile surgical precautions being employed. After intervals of hydronephrosis of from one to forty-two days, 9 rabbits were given subcutaneously 5 mg. per kilogram of a 0.5 per cent aqueous solution of uranium nitrate. Five or six days later the animals were killed. The left ureter of 1 rabbit had been partially obstructed by tying a wire 0.9 mm. in diameter alongside the ureter, removing the wire and leaving the tie in place. In rabbits with unilateral hydronephrosis of five days' duration or longer the hydronephrotic kidney was protected against severe uranium injury, while the proximal convoluted tubules of the other kidney were destroyed or greatly damaged. The single rabbit with high grade partial obstruction revealed the same protection (table 1).

TABLE 1.—*Nephrotoxic Effect of Uranium Nitrate Injected Subcutaneously in the Presence of Unilateral Hydronephrosis*

Rabbit	Duration of Hydronephrosis Before Injection, Days	Dose of Uranium Nitrate, Mg.	Duration of Life Following Injection, Days	Nephrotoxic Effect	
				Right Kidney	Left Kidney (Hydronephrotic)
R-1	1	10.0	6	+++	+++
R-2	2	10.0	3*	++++	+++
R-3	3	9.0	4	++	++
R-4	4	8.0	6	+++	+++
R-5	5	10.0	4*	+++	+
R-6	6	14.0	6	+++	+
R-7	10	12.0	5	+++	+
R-8	14	12.5	6	++++	+
R-9	42	15.0	6	++++	+
R-10	8†	10.0	6*	+++	+

* The rabbit was found dead.
† The left kidney was subjected to high grade partial obstruction.

Five rabbits with hydronephrosis of from one to twenty-eight days' duration were given a subcutaneous

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1. Wilmer, H. A.: J. Exper. Med. **78**:225, 1943.
2. Frola, E.: Pathologica (*a*) **20**:279, 1928; (*b*) **21**: 109, 1929.
3. Clivio, cited by Frola.[2a]
4. MacNider, W. deB.: J. Exper. Med. **23**:171, 1916.
5. Goto, K.: J. Exper. Med. **25**:693, 1917.
6. Donnelly, G. L., and Holman, R. L.: J. Pharmacol. & Exper. Therap. **75**:11, 1942.
7. Havill, W. H.; Lichty, J. A., Jr., and Whipple, G. H.: J. Exper. Med. **55**:627, 1932.
8. Selye, H.: J. Pharmacol. & Exper. Therap. **68**: 454, 1940.
9. Holman, R. L., and Donnelly, G. L.: J. Exper. Med. **76**:511, 1942.
10. Wilmer, H. A.: Am. J. Physiol. **141**:431, 1944.

injection of racemic tartaric acid neutralized with sodium bicarbonate as described by Underhill, Wells and Goldschmidt[11]; a dose of 0.51 to 0.68 Gm. of a 17 per cent aqueous solution was used, and, as in the experiments of Potter and Bell,[12] the animals were killed within seven hours after injection of the acid. In both the hydronephrotic kidney and the kidneys which had been left unobstructed severe hydropic degeneration of equal intensity was present at all intervals.

The nephrotoxic effect of mercury bichloride was determined in 5 rabbits with hydronephrotic left kidneys after one to thirty days' obstruction. These animals had been given 4.0 to 7.5 mg. of a 1:1,000 aqueous solution of mercury bichloride. Rabbit 16, which received the poison immediately after the ligation of the ureter, was found dead in twenty-four hours. After an interval of obstruction of only twenty-four hours the hydronephrotic kidney was protected from the severe destructive tubular injury of mercury bichloride (table 3).

TABLE 2.—*Nephrotoxic Effect of Racemic Tartaric Acid Injected Subcutaneously in the Presence of Unilateral Hydronephrosis*

Rabbit	Duration of Hydronephrosis, Days	Dose of Racemic Acid, Gm.	Duration of Life Following Injection, Hours	Nephrotoxic Effect: Right Kidney	Nephrotoxic Effect: Left Kidney (Hydronephrotic)
R-11	1	0.51	7	++++	++++
R-12	7	0.51	7	++++	++++
R-13	14	0.68	5	++++	++++
R-14	21	0.68	.	++++	(*)
R-15	28	0.59	5	++++	++++

* There was severe pyelonephritis; the remaining tubules were not damaged.

TABLE 3.—*Nephrotoxic Effect of Mercury Bichloride Injected in the Presence of Unilateral Hydronephrosis* *

Rabbit	Duration of Hydronephrosis Before Injection, Days	Dose of Mercury Bichloride, Mg.	Duration of Life Following Injection, Days	Nephrotoxic Effect: Right Kidney	Nephrotoxic Effect: Left Kidney (Hydronephrotic)
R-16	Immediate	7.5	1	++++	++++
R-17	1	4.0	5	+++	+
R-18	1	5.0	3	+++	+
R-19	2	5.0	5	+++	+
R-20	30	5.0	2	++++	Trace

* Dr. Douglas Adkins prepared these animals.

Diethylene glycol was administered to 3 rabbits with unilateral hydronephrosis of one, seven, and thirty days' duration. A dose of 8 to 9 cc. was injected intramuscularly.[13] In all 3 animals diethylene glycol produced hydropic degeneration of equal intensity in both the hydronephrotic kidney and the kidney with ureter unsevered (table 4).

TABLE 4.—*Nephrotoxic Effect of Diethylene Glycol Injected Intramuscularly in the Presence of Unilateral Hydronephrosis* *

Rabbit	Duration of Hydronephrosis Before Injection, Days	Dose of Diethylene Glycol, Cc.	Duration of Life After Injection, Days	Nephrotoxic Effect: Right Kidney	Nephrotoxic Effect: Left Kidney (Hydronephrotic)
R-21	1	8.0	2	++++	++++
R-22	7	8.5	2	++++	+
R-23	30	9.0	2	++++	++++

* Dr. Douglas Adkins prepared these animals.

In each series of experiments the most intense injury was graded as 4 plus.

One rabbit which had been pregnant fourteen days was given subcutaneously 5 cc. of a 1:1,000 solution of mercury bichloride and was killed five days later. The maternal kidneys were severely damaged while the fetal kidneys were unaffected.

COMMENT

Uranium nitrate produces coagulation necrosis like a true protoplasmic poison. There seems to be a selective affinity for the proximal convoluted tubules. When the ureter of the left kidney is doubly tied and severed and an interval of five days or more allowed to pass before uranium nitrate is administered, the hydronephrotic kidney is protected from severe tubular injury, while the right kidney shows necrosis of the epithelium of the proximal convoluted tubules, often so intense that the entire tubule is filled with a homogeneous eosin-staining mass of necrotic debris. The hydronephrotic kidney shows desquamation and necrosis in a few tubules (figs. 1 and 2). Glomerular changes in the animals observed in this study were scattered and slight, although occasionally a hemorrhagic glomerular lesion stood out in a field of unaffected tubules in the hydronephrotic kidney. The animals had polyuria, albuminuria and often glycosuria.

Mercury bichloride, like uranium nitrate, is a true protoplasmic poison and produces coagulation necrosis of the proximal convoluted tubules, although the histologic alterations are somewhat different. Necrosis is often limited to the luminal portion of the cells, so that the inner part of the cell desquamates, leaving only a basal layer of the epithelium. Under these circumstances the blood colloids, unimpeded by the normal qualities of the tubular epithelium, are able to draw back the entire glomerular filtrate, with resulting anuria.[14] After only twenty-four hours of ureteral obstruction the hydronephrotic tubules are protected against severe damage by mercury

11. Underhill, F. P.; Wells, H. G., and Goldschmidt, S.: J. Exper. Med. **18**:317, 1913.

12. Potter, A. C., and Bell, E. T.: Am. J. M. Sc. **149**:236, 1915.

13. (*a*) Geiling, E. M. K.; Coon, J. M., and Schoeffel, E. W.: J. A. M. A. **109**:1532, 1937. (*b*) Geiling, E. M. K., and Cannon, P. R.: ibid. **111**:919, 1938.

14. Richards, A. N.: Tr. A. Am. Physicians **44**:64, 1929.

bichloride. Calcification of the collecting tubules occurred in the right kidney, which was not subjected to surgical interference, but not in the hydronephrotic left kidney (figs. 3 and 4).

Diethylene glycol and racemic acid, like sucrose, produce hydropic degeneration of the convoluted tubules, limited almost exclusively to the proximal segment, although with the first two drugs slight disturbance of the distal segment may be evident. The hydropic degeneration induced by diethylene glycol is coarsely vacuolar, progresses eventually to necrosis and results in uremia. Geiling and Cannon [13b] described the histologic change as a ballooning of the convoluted tubular epithelium with "water-logged" cells, which mechanically block the lumen. They expressed the opinion that the effects were not those of an extremely toxic protoplasmic poison. Diethylene glycol is a hygroscopic agent—presumably because of the two hydroxyl groups in its molecule. The drug might thus produce intracellular edema, or since it is an ether, its solvent powers might produce injury of the cell membrane followed by imbibition of water.[13b] Persons killed in St. Louis by ingestion of a diethylene glycol elixir of a sulfonamide compound showed hydropic degeneration of the epithelium of the convoluted tubules with desquamation, tubular necrosis, hemorrhage and obstruction of the tubules by casts.[13a] In the rabbits diethylene glycol produced severe and equal hydropic degeneration in both the hydronephrotic kidney and the one with unaltered ureter at the intervals studied up to one month. Only forty-eight hours was required for tubular damage with enormous cytoplasmic vacuolation.

Just as in diethylene glycol poisoning so in poisoning with racemic tartaric acid hydropic degeneration occurred in both the hydronephrotic kidney and the kidney the ureter of which was not operated on, at all intervals studied up to twenty-eight days. This drug provokes tubular injury within two to five hours,[12] and by the end of six days there is complete destruction of the epithelium of the convoluted tubules, so that Underhill, Wells and Goldschmidt [11] suggested that tartaric acid is eliminated by the convoluted tubules. This explanation is not necessarily correct. Taking advantage of the selective necrosis of the proximal convoluted tubules caused by racemic acid, Friedman and Kaplan [15] determined

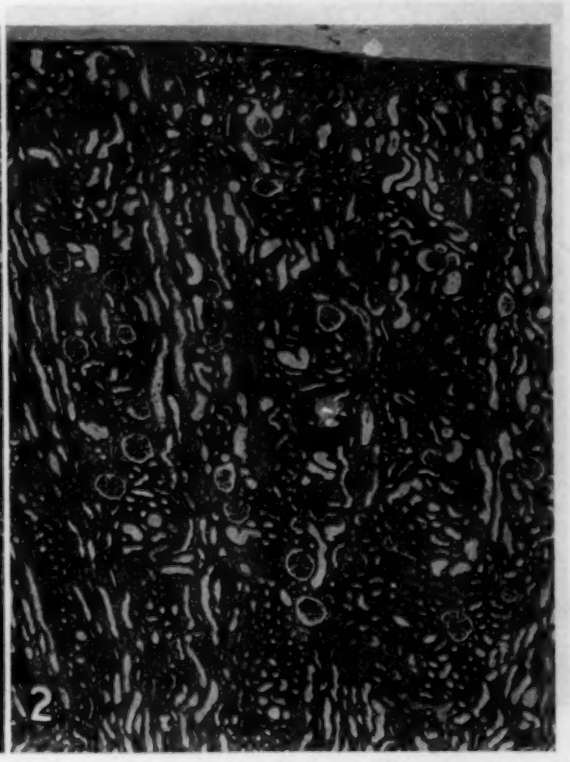

Fig. 1.—Right kidney of rabbit 7, which had hydronephrosis of the left kidney of ten days' duration due to ureteral obstruction on the left. The animal was killed five days after administration of uranium nitrate. The photomicrograph (× 35) shows extensive tubular destruction.

Fig. 2.—The hydronephrotic left kidney from the same rabbit, showing almost complete protection from uranium nitrate injury. Photomicrograph; × 35.

15. Friedman, M., and Kaplan, A.: J. Exper. Med. **77**:65, 1943.

the renin content of the kidney following experimental poisoning and concluded that renin is either produced or stored (or both) in the proximal segment. The hydropic lesions are coarsely vacuolar.

While racemic tartaric acid and diethylene glycol both injure the hydronephrotic kidney, uranium nitrate and mercury bichloride fail to do so.

HYPOTHESES

Profound functional changes occur in the hydronephrotic kidney: Phosphatase disappears [1]; the rate of renal respiration is depressed [16] and the organ cannot elaborate concentrated urine.[17] Now, in view of the fact that mercury bichloride and uranium nitrate produce coagulation necrosis while diethylene glycol, racemic acid and sucrose provoke a hydropic type of injury, one might assume that the slower acting protoplasmic poisons require prolonged exposure in a concentrated glomerular filtrate and that the more rapidly produced hydropic degeneration is the result of osmotic disturbance or of hygroscopic action of the drugs, which diffuse rapidly into the highly susceptible epithelium of the proximal convoluted tubules. Presumably, these drugs exert their nephrotoxic action from the lumen. It is conceivable that racemic acid and diethylene glycol are partially eliminated by the tubular epithelium and injure the cells from the blood stream. They could thereby exert their injurious effect in the absence of a concentrated filtrate; on the other hand, it may be that small quantities in the glomerular filtrate are highly toxic to the epithelium of the proximal segment. Both of these hypotheses may well be incorrect, for sucrose, which likewise injures the hydronephrotic kidney, does so only in large concentrations from the lumen.[10] Unlike the hydropic degeneration caused by the other two drugs, however, that produced by sucrose does not lead to necrosis.

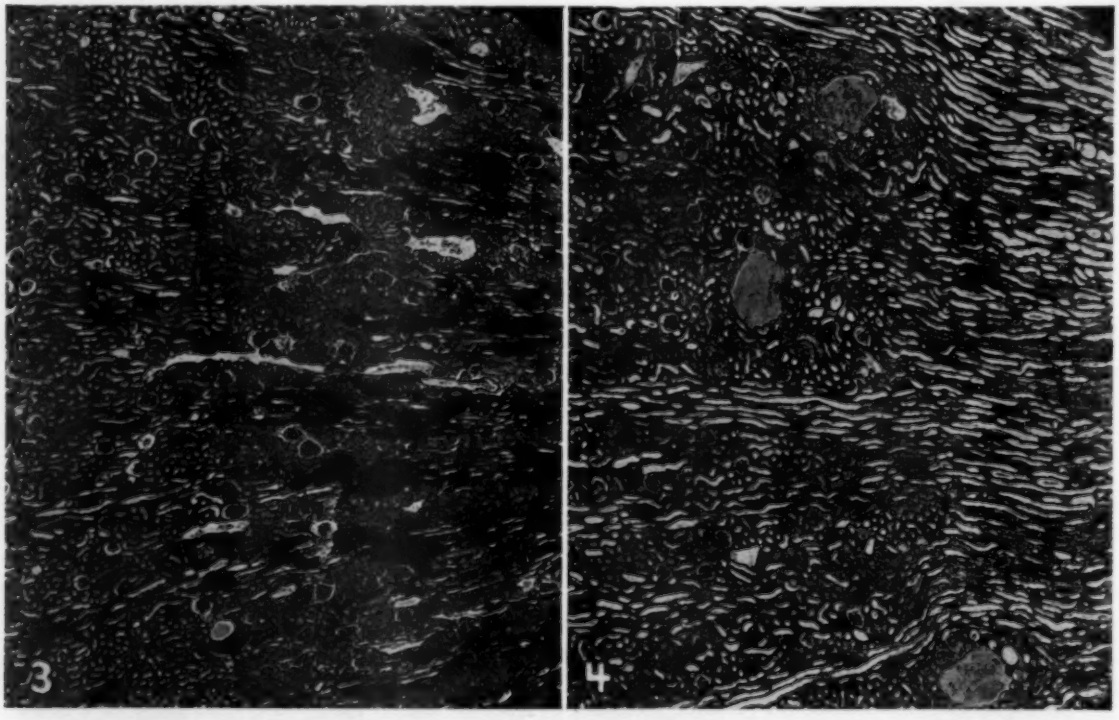

Fig. 3.—Right kidney from rabbit 17, which had hydronephrosis of the left kidney of one day's duration before poisoning. The animal was given mercury bichloride and was killed five days later. Note the tubular injury and the calcification of collecting tubules. Photomicrograph; × 29.

Fig. 4.—The hydronephrotic left kidney from the same rabbit, showing almost complete protection. Note the absence of calcification. Photomicrograph; × 29.

Some minimal injury is always found in hydronephrotic kidneys poisoned with uranium or mercury. It is therefore evident that the cells do not completely exclude these poisons. To explain why two of the four drugs studied are nephrotoxic and two are not, the following explanation is advanced: The hydronephrotic process produces rapid tubular disturbances and

16. Wilmer, H. A.: Arch. Path. **37**:227, 1944.

17. Wilmer, H. A.: Proc. Soc. Exper. Biol. & Med. **56**:52, 1944.

leaves the glomeruli unaffected until late. The intrapelvic pressure is sufficient to suppress but not arrest glomerular filtration.[17] When the ureteral obstruction of a hydronephrotic kidney is released only a small quantity of urine of low specific gravity is excreted.[18] Glomerular filtration occurs and probably most of the filtrate is rapidly reabsorbed (or diffuses) through the convoluted tubules. Concentration does not occur. Presumably, therefore, uranium and mercury are not nephrotoxic unless concentrated in the tubule, and diethylene glycol and racemic acid are nephrotoxic in the concentration of the glomerular filtrate as in the case of sucrose. The evidence in favor of this hypothesis is not conclusive.

SUMMARY

In the rabbit, after a period of complete unilateral ureteral obstruction of five days or more the hydronephrotic kidney is protected against the nephrotoxic action of uranium nitrate, while after a period of only twenty-four hours the hydronephrotic kidney is protected against severe destruction (and later calcification) induced by mercury bichloride.

Diethylene glycol and racemic tartaric acid produce intense hydropic degeneration and necrosis in both kidneys of the rabbit with unilateral hydronephrosis up to one month's duration.

It is suggested as a possible explanation that the slower acting protoplasmic poisons—uranium nitrate and mercury bichloride—are not injurious to the hydronephrotic kidney because they require a concentration found only in the presence of normal filtration-reabsorption, while diethylene glycol and racemic tartaric acid are nephrotoxic in the absence of concentration of the glomerular filtrate in hydronephrosis.

18. Johnson, R. A.: J. Exper. Med. **28**:193, 1918.

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FACTORS INFLUENCING THE PERSISTENCE OF VITAMIN A FLUORESCENCE IN TISSUE SECTIONS

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Frozen sections of human and animal tissue (liver, cortex of adrenal gland and other organs) display under the fluorescence microscope a green, quickly fading fluorescence which has been utilized for the microscopic visualization of vitamin A.[1] This fluorescence disappears quickly when the frozen sections are kept in water. The speed of disappearance is faster in animal than in human organs and varies with different persons and different locations of the fluorescence in the organ.[2] In recent years the question of destruction of vitamins became significant in the explanation of the endogenous or conditioned vitamin deficiency.[3] Various authors described vitamin B deficiency as brought on by destruction of the vitamin in the organism after its absorption from the intestinal tract.[4] Destruction of vitamin A or carotene by oxidation [5] or by substances occurring in the human or the animal body has been described. Such substances are linolenates and linolates,[6] palm oil and decolorized butter fat,[7] rancid fat,[8] stale diets [9] and emulsions of tissues.[10] The significance of this destruction in the bioassay and in the evaluation of requirements has been stressed.[11] The factors influencing or preventing this destruction are little known except for the antioxidative effect of tocopherol (vitamin E).[12] With this problem in mind, the factors which influence the speed of disappearance of vitamin A fluorescence from tissue sections were studied to obtain information as to the conditions which may influence the destruction of vitamin A in the body.

MATERIAL AND METHODS

Frozen sections of livers and adrenal glands obtained from rats and from patients who had died from various diseases were kept in water or isotonic solution of sodium chloride for twenty-four hours. Their vitamin A fluorescence was examined by means of fluorescence microscopy; the method used was discussed in detail in a previous publication.[2] The fluorescence was compared with that present in sections of the same specimen kept for the same period in various mediums, some of biologic nature. The sections were kept at room temperature or at temperatures of 6 C. and 37 C. The sections kept in water or saline solution served as controls for the speed of disappearance of the vitamin A fluorescence. Attention was paid not only to the total amount but also to the different localizations of the vitamin A fluorescence, particularly in the sections of liver.

Plasma in different forms, other biologic fluids, emulsions of organs and some nonbiologic materials were used as test mediums. In order to determine the lowest concentration in which the speed of disappearance of the fluorescence was equal to that in water or saline solution, different concentrations of plasma were used. Plasma was also subjected to various procedures to establish the qualities of the factor which produces the difference in the speed of disappearance of the fluorescence from sections kept in plasma and in water or saline solution. In addition, the influence of various dietary measures on this factor in plasma was studied. The emulsions of organs were prepared by crushing the organs in a mortar; the crushed material was then suspended in saline solution.

The difference of the amount of vitamin A fluorescence which had disappeared from the tissue sections in a given medium from that which disappeared in water or saline solution was indicated by plus signs if more disappeared in water and by minus signs if less disappeared in water. The number of symbols indicated the degree of difference.

From the Hektoen Institute for Medical Research of the Cook County Hospital.

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RESULTS

Comparison of the Disappearance of Vitamin A Fluorescence in Human and in Animal Tissues.—Vitamin A fluorescence disappeared faster from animal than from human tissues (table 1); it disappeared from the adrenal glands slower than from the liver, both in animals and in man. In sections of liver the vitamin A fluorescence vanished much faster from the Kupffer cells than from the liver cells. In the analysis shown in table 1, human liver was taken as an example, and in every case the average of the vitamin A fluorescence of the Kupffer and the liver cells was used in the comparison.

TABLE 1.—*Disappearance of Vitamin A Fluorescence from Human and Animal Tissues* *

	Liver			Adrenal Gland	
	Cases	Kupffer Cells	Hepatic Cells	Cases	Epithelial Cells
Man	110	++	+	8	+
Rats	15	+++	++	5	++

* Plus signs indicate the comparative speed of disappearance.

TABLE 2.—*Influence of Different Biologic Mediums on the Disappearance of Vitamin A Fluorescence from Tissue Sections*

Medium	Vitamin A Fluorescence in Comparison with Sections Kept in Water*	Cases	Medium	Vitamin A Fluorescence in Comparison with Sections Kept in Water*	Cases
Plasma	++++	92	Suspension of white blood cells	++	3
Serum	++++	10	Urine	++	34
Emulsion of liver	+++	17	Ascites	+++	10
Emulsion of pancreas	++	2	Spinal fluid	++	15
Emulsion of spleen	++	4	Bile	+++	5
Emulsion of thyroid	++	2	Lecithin	++	11
Emulsion of lung	++	2	4% solution of formaldehyde	—	12
Emulsion of kidney	++	5	Ammonium hydroxide	——	5
Emulsion of myocardium	+	10	Hydrogen peroxide	————	15

* The difference of the amount of vitamin A fluorescence which had disappeared from the tissue sections in a given medium from that which disappeared in water is indicated by plus signs if more disappeared in water and by minus signs if less disappeared in water.

Influence of Various Biologic Mediums and Nonbiologic Substances on the Disappearance of Vitamin A Fluorescence.—Most of the biologic mediums examined preserved vitamin A fluorescence better than water or saline solution (table 2). Plasma and serum head the list of the mediums which delayed the disappearance of vitamin A fluorescence. Other biologic fluids and emulsions of organs were less efficient. Of the nonbiologic substances examined, only lecithin appeared to have a similar effect. A 44 per cent solution of formaldehyde and ammonia water both had a slight destroying effect. The strongest destroying effect was exerted by hydrogen peroxide.

Influence of Temperature on the Disappearance of Vitamin A Fluorescence.—The disappearance of vitamin A fluorescence is more pronounced at room temperature than at ice box or incubator temperature. This difference, however, was not conspicuous in all mediums (table 3).

Influence of Various Procedures on the Ability of Plasma to Delay the Disappearance of Vitamin A Fluorescence from Tissue Sections.—Plasmas of various patients were submitted to different procedures in an attempt to separate the active principle which delays the disappearance of vitamin A fluorescence. The

TABLE 3.—*Influence of Temperature on the Disappearance of Vitamin A Fluorescence from Tissue Sections*

Medium	Cases	Vitamin A Fluorescence Present in Tissue Sections Kept at 37 C.	Room Temperature	Ice Box Temperature
Plasma	92	+++	++	++++
Boiled plasma	11	+++	++	++++
Emulsion of liver	17	+++	++	++++
Emulsion of kidney	5	++	++	+++
Emulsion of pancreas	2	++	++	++
Emulsion of spleen	4	++	++	++
Emulsion of thyroid	2	++	++	+++
Emulsion of myocardium	10	+	±	+
Emulsion of lung	2	+	+	++
Suspension of white blood cells	3	++	++	++
Lecithin	11	++	++	+++

TABLE 4.—*Influence of Various Procedures on the Ability of Plasma to Delay the Disappearance of Vitamin A Fluorescence from Tissue Sections*

Medium	Cases	Vitamin A Fluorescence Present in Comparison with Sections Kept in Water
Plasma	92	++++
Boiled plasma	11	+++
Putrid plasma	5	+++
Digested plasma	4	+++
Dialyzed plasma	13	++
Plasma extracted with ether	5	+++

amount of the active principle present after treatment was compared with that present before treatment. Table 4 shows the average of all plasmas submitted to a given treatment. Boiling of plasma in the dilution of 1:3, spontaneous putrefaction, digestion with pancreatic enzymes or extraction of the undiluted plasma with ethyl ether reduced the active principle only slightly. Dialysis reduced it significantly without abolishing it completely.

Influence of Various Physiologic and Pathologic Conditions on the Ability of Plasma to Delay the Disappearance of Vitamin A Fluorescence.—There was no definite relation between the vitamin A level of the plasma and its ability to delay the disappearance of vitamin A fluorescence (table 5). Plasmas with low vitamin A—below 10 micrograms per hundred cubic centimeters—

showed the same activity as plasmas with concentrations above 60 micrograms per hundred cubic centimeters. Plasmas from patients with pneumonia, cirrhosis and acute hepatitis did not differ from others. Administration of 50 mg. of mixed tocopherol[13] for three days was of no influence. The active principle appeared slightly reduced two hours after a meal if compared with that present in the fasting state.

TABLE 5.—*Influence of Various Physiologic and Pathologic Conditions on the Ability of Plasma to Delay the Disappearance of Vitamin A Fluorescence from Tissue Sections*

Condition	Cases	Vitamin A Fluorescence Present in Comparison with Sections Kept in Water	Condition	Cases	Vitamin A Fluorescence Present in Comparison with Sections Kept in Water
Pneumonia.....	5	++++	Low level of plasma vitamin A	5	++++
Cirrhosis.....	4	++++	Mixed tocopherol had been administered	5	++++
Acute hepatitis..	3	++++	Patients were fasting	10	++++
High level of plasma vitamin A.....	5	++++	Plasma examined two hours after meals	10	+++

Titration of the Active Principle.—In 35 cases diluted plasmas (1:2, 1:4, 1:8, 1:16 and 1:32) were examined for their ability to delay the disappearance of vitamin A fluorescence. The active principle was found in plasma diluted up to 1:32 in some cases; usually, however, only in that diluted to 1:8. No relation of this titer to diseases or to concentration of vitamin A in the plasma was found.

COMMENT

In various biologic fluids an active principle is found which delays the disappearance of vitamin A fluorescence from tissue sections. Fat stains show that this disappearance concerns only vitamin A fluorescence and not the fat which carries vitamin A. This active principle may thus be considered as protecting vitamin A itself.

Some of the characteristics of this principle have been determined. It is present in highest amounts in plasma. Its amount does not depend on disease or on the concentration of vitamin A. The protective principle is apparently reduced by intake of food. Its activity is influenced by the temperature. It is, however, not destroyed by boiling. It is not fat soluble and is not destroyed by digestion or putrefaction. It seems to be partly dialyzable. In addition to being present in plasma it occurs in lower concentrations in emulsions of organs and in various biologic fluids. The nature of this protective principle, however, is not clear.

There are various substances which enhance the disappearance of vitamin A fluorescence from tissue sections, such as hydrogen peroxide.

Whether the described principle, protective in vitro, has any biologic significance in vivo, is not evident. This question is interesting at the present time, when in addition to exogenous nutritional vitamin deficiencies, the importance of endogenous vitamin A deficiency is stressed.[14] One of the factors in endogenous vitamin A deficiency may be increased destruction of vitamin A in the body. One could speculate that the presence or lack of a protective principle in the tissues may influence the vitamin A metabolism and the vitamin A stores of the body, its absence causing endogenous vitamin A deficiency.

SUMMARY

In plasma, in other biologic fluids and in emulsions of organs an active principle was found which delays the disappearance of vitamin A fluorescence of tissue sections as compared with those kept in water or saline solution. This protective principle is not influenced by diseases or by the level of vitamin A in the plasma. Its activity depends on the temperature. It is not destroyed by boiling, digestion or putrefaction and is partly dialyzable. Whether this protective principle has any biologic significance is questionable.

13. The tocopherol was supplied by Distillation Products, Inc., Rochester, N. Y.

14. Popper, H., and Steigmann, F.: J. A. M. A. **123**:1108, 1943. Moore, T.: Post-Grad. M. J. **17**:52, 1941. Thiele, W.: Klin. Wchnschr. **19**:1201, 1940. Joliffe.[3]

EXPERIMENTAL STUDIES ON THE THERAPY AND PREVENTION OF DEGENERATIVE VASCULAR DISEASES

I. THE EFFECT OF MEDICATION WITH POTASSIUM THIOCYANATE ON EXPERIMENTAL CHOLESTEROL ATHEROMATOSIS

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Thiocyanates have attracted attention during recent years mainly for three reasons: (1) They have been used more or less successfully in the treatment of hypertension because of their blood pressure–lowering action; (2) they have been employed as agents preventing or impairing the development of experimental cholesterol atherosclerosis in rabbits, and (3) they have been shown to interfere with the functional activity of the thyroid gland, giving rise to the production of hypothyroidotic goiter. Any one of the three activities listed establishes connections between thiocyanates and degenerative vascular diseases. By lowering the blood pressure and by interfering with the deposition of cholesterol in the arterial walls, thiocyanates may counteract the development of such vascular disorders. The demonstrated impairment of the thyroid secretion, on the other hand, places these agents in a role which should favor the production of degenerative vascular lesions because of the secondary appearance of a hypothyroidotic hypercholesteremia, which is associated with a lowering of the blood iodine level and of the basal metabolic rate.

Inasmuch as previous investigations on the arteriosclerotogenic aspects of thiocyanates had been made without regard to these antagonistic properties, a reexamination of this problem appeared to be indicated, with proper consideration given to the hematic, organic and vascular reactions elicited by the thiocyanates.

EXPERIMENTAL PROCEDURE

Three dogs and 12 rabbits were used in the experiment. The dogs, which weighed 9.5 Kg., 10 Kg. and 11.5 Kg., respectively, received daily 20 mg. of potassium thiocyanate given as a 10 per cent solution in milk. After five weeks the dose was increased to 30 mg.; after twelve weeks, to 60 mg., and after fifteen weeks, to 100 mg. It was kept at this level for five weeks, after which the animals were killed by intravenous injection of a 4 per cent formaldehyde solution. As the dogs were kept on prepared dog chow, any reactions observed in them would represent those elicited by thiocyanates in omnivorous animals.

The rabbits, about 3 months old and weighing from 2.2 Kg. to 2.7 Kg., were divided into two groups. Seven rabbits received daily in the morning 20 cc. of water containing 6 mg. of potassium thiocyanate. A second series of 5 rabbits was given the same dose of potassium thiocyanate and 0.25 Gm. of cholesterol in 10 cc. of corn oil. The oil was mixed with a small amount of rabbit chow, which the rabbits had to consume before any additional food was offered. The dose of thiocyanate was increased after five weeks by 50 per cent and after seven weeks by 100 per cent. The dose was so adjusted that the level of the blood thiocyanate was stabilized at about 5 mg. per hundred cubic centimeters of blood for fourteen weeks; administration of the drug was then discontinued for five weeks and later resumed. This level of medication was maintained until the end of the experiment. Three rabbits in the first series died during the course of the experiment after ten days, three weeks and nine weeks, respectively.

The feeding of cholesterol and thiocyanate was discontinued after fourteen weeks. The treatment with thiocyanate was resumed after a five week interval and continued until the survivors were killed. Two of each series were killed by an intravenous injection of formaldehyde solution sixteen weeks after the start of the experiment, while the remaining rabbits were killed by the same method twenty-two weeks later.

HEMATIC STUDIES

The hematic studies included for every animal the following determinations: the hemoglobin content per hundred cubic centimeters, the number of erythrocytes and the number of leukocytes per cubic millimeter, the sedimentation rate, the viscosity of the plasma, the blood sugar level, the serum cholesterol level and the thiocyanate content of the serum. These determinations were made at weekly intervals. The blood was withdrawn from the jugular vein in both species.

Dogs.—The hematic changes in the 3 dogs followed a uniform pattern, which is illustrated in table 1, giving the hematic data of 1 representative dog.

All 3 dogs showed a moderate increase in the amount of hemoglobin toward the end of the experiment. The numbers of erythrocytes and leukocytes, the sedimentation rate and the viscosity of the plasma did not undergo any consistent changes. The serum cholesterol and the blood sugar exhibited a definite tendency to rise during the latter part of the experimental period, remaining in general in the high normal or low pathologic range.

Rabbits.—(*a*) Thiocyanate Series: The hematic changes in the 7 rabbits composing this series followed in general outlines the pattern which is illustrated in table 2, showing the data of 1 animal.

The rabbits showed a mild to moderate increase in the amount of hemoglobin toward the end of the experimental period. The numbers of erythrocytes and

From the Warner Institute for Therapeutic Research.

leukocytes, the sedimentation rate and the viscosity of the plasma did not undergo any consistent changes. Serum cholesterol and blood sugar values revealed, on the other hand, a progressive and moderate elevation. The cholesterol level during the latter part of the experimental period was definitely in a range which is abnormally high for rabbits.

(*b*) Thiocyanate-Cholesterol Series: The hematic reactions observed in this series are illustrated in table 3, which gives the data obtained on 1 representative animal.

The rabbits showed a moderate increase in the amount of hemoglobin starting during the first weeks of the experiment and being maintained through almost the entire period. There were no significant changes in the numbers of erythrocytes and leukocytes and the viscosity of the plasma. The serum cholesterol was definitely increased as long as the cholesterol was given but quickly dropped back to the normal level after the administration of cholesterol was discontinued. The level of thiocyanate remained elevated somewhat longer than that of cholesterol after the cessation of treatment and rapidly reached abnormal values again after the resumption of the thiocyanate treatment.

The increase in serum cholesterol in these rabbits was of a magnitude similar to that seen in a series of 5 rabbits which received 0.25 Gm. of cholesterol daily in 10 cc. of corn oil in another experimental study. The administration of potassium thiocyanate therefore had apparently no effect in either direction on the level of the serum cholesterol.

TABLE 1.—*Hematic Reactions in Dog 1386 Following Prolonged Administration of Potassium Thiocyanate*

Weeks	Hemoglobin, Gm.	Erythrocytes, Millions	Leukocytes, Thousands	Sedimentation Rate, Mm.	Viscosity of Plasma	Serum Cholesterol, Mg.	Blood Sugar, Mg.	Thiocyanate in Serum, Mg.
0	14.4	8.2	17.4	2	2.0	141.5	102.5	0
1	15.1	8.1	10.2	0	2.25	114.0	125.5	1.25
2	17.8	6.9	15.8	0	2.3	148.5	116.5	3.48
3	16.3	6.7	17.2	1	1.65	192.0	156.0	4.78
4	17.75	5.3	13.4	0	1.7	168.0	176.9	4.65
6	18.7	7.2	14.2	0	1.8	192.0	172.0	4.8
7	20.2	6.7	12.1	0	1.8	132.0	181.0	5.1
8	19.2	7.1	12.3	0	1.85	149.5	176.8	5.1
11	18.7	7.6	14.2	0	1.95	200.0	146.5	4.6
12	19.2	9.7	18.4	0	1.85	184.0	170.0	4.9

TABLE 2.—*Hematic Reactions in Rabbit 38 Following Prolonged Administration of Potassium Thiocyanate*

Weeks	Hemoglobin, Gm.	Erythrocytes, Millions	Leukocytes, Thousands	Sedimentation Rate, Mm.	Viscosity of Plasma	Serum Cholesterol, Mg.	Blood Sugar, Mg.	Thiocyanate in Serum, Mg.
0	14.7	8.1	11.4	0	1.5	124	.....	0.25
1	15.4	6.3	5.0	0	1.7	76.7	130.5	0
2	15.9	5.8	5.4	0	1.6	106.1	146.2	4.6
3	15.3	7.1	9.8	0	1.6	143.5	153.5	4.3
4	16.3	7.5	9.0	0	1.6	107.0	158.0	4.2
5	13.5	7.1	7.6	0	1.7	139.5	179.0	4.2
6	14.2	8.0	10.4	0	1.9	180.0	177.0	4.38
11	16.3	6.6	13.8	0	2.0	169.5	198.0	4.55

TABLE 3.—*Hematic Reactions in Rabbit 42 Following Prolonged Administration of Potassium Thiocyanate and Cholesterol*

Weeks	Hemoglobin, Gm.	Erythrocytes, Millions	Leukocytes, Thousands	Sedimentation Rate, Mm.	Viscosity of Plasma	Serum Cholesterol, Mg.	Blood Sugar, Mg.	Thiocyanate in Serum, Mg.
0	9.35	6.8	8.1	0	1.55	119.0	.....	1.4
1	12.5	5.6	11.4	0	1.75	297.0	130.0	4.8
2	15.4	5.0	9.8	0	2.0	435.0	140.0	5.0
3	15.2	4.9	8.6	0	1.6	365.0	136.0	4.3
4	14.4	5.5	10.6	0	1.65	204.5	190.5	4.6
5	14.7	5.8	8.4	0	1.6	299.5	155.5	4.7
6	13.43	6.2	12.8	0	1.45	192.0	167.0	3.9
7	14.4	6.0	9.0	0	1.5	302.0	144.0	4.7
11	15.35	5.5	14.8	0	1.7	319.0	195.0	4.6
15*	....	...	....	..	...	178.0	.....	4.3
19†	13.0	6.0	8.4	0	1.7	79.5	139.5	0.4
21	12.9	5.9	10.1	0	1.6	95.6	145.0	6.8

* This was one week after the discontinuation of the administration of potassium thiocyanate and cholesterol.
† This was one week before the resumption of the administration of potassium thiocyanate.

PATHOLOGIC STUDIES

The postmortem examination of the dogs did not reveal any abnormal gross changes. The thyroid glands were of normal size and pale brown to red brown. The livers were congested as the result of the intravenous injection of formaldehyde solution.

The histologic examination of the thyroid glands of the dogs showed two to be composed of large follicles filled with solid, deep pink-stained colloid, while in the third about one third of the follicles were of small to medium size and were either empty or filled with thin colloid. The anterior lobe of the hypophysis of this

animal was congested and consisted mainly of eosinophilic cells. The ascending aorta of 1 dog exhibited a markedly edematous inner zone of the media and a large intimal cushion of fibrohyaline tissue (fig. 1 *A*). In the thoracic aorta of this dog and in one of its large branches an edematous media was covered by a few loose fibrillar intimal thickenings. Similar medial changes located beneath an albuminous intimal cushion were present in the aorta of the second dog (fig. 1 *B*), while the outer zone of the media contained two large hyaline and partly calcified hyaline areas. The aorta of the third dog was normal, but one of its smaller branches revealed small intimal hyaline thickenings (fig. 2 *A*). The splenic follicular arterioles of the first dog had thickened hyaline walls.

The rabbits of both series showed at autopsy small to normal-sized, pale brown-red thyroid glands and ample amounts of retroperitoneal and peritoneal fat tissue. One of the rabbits of the potassium thiocyanate series died with congested and hemorrhagic lungs, while a second rabbit of this series died with hemorrhagic duodenitis.

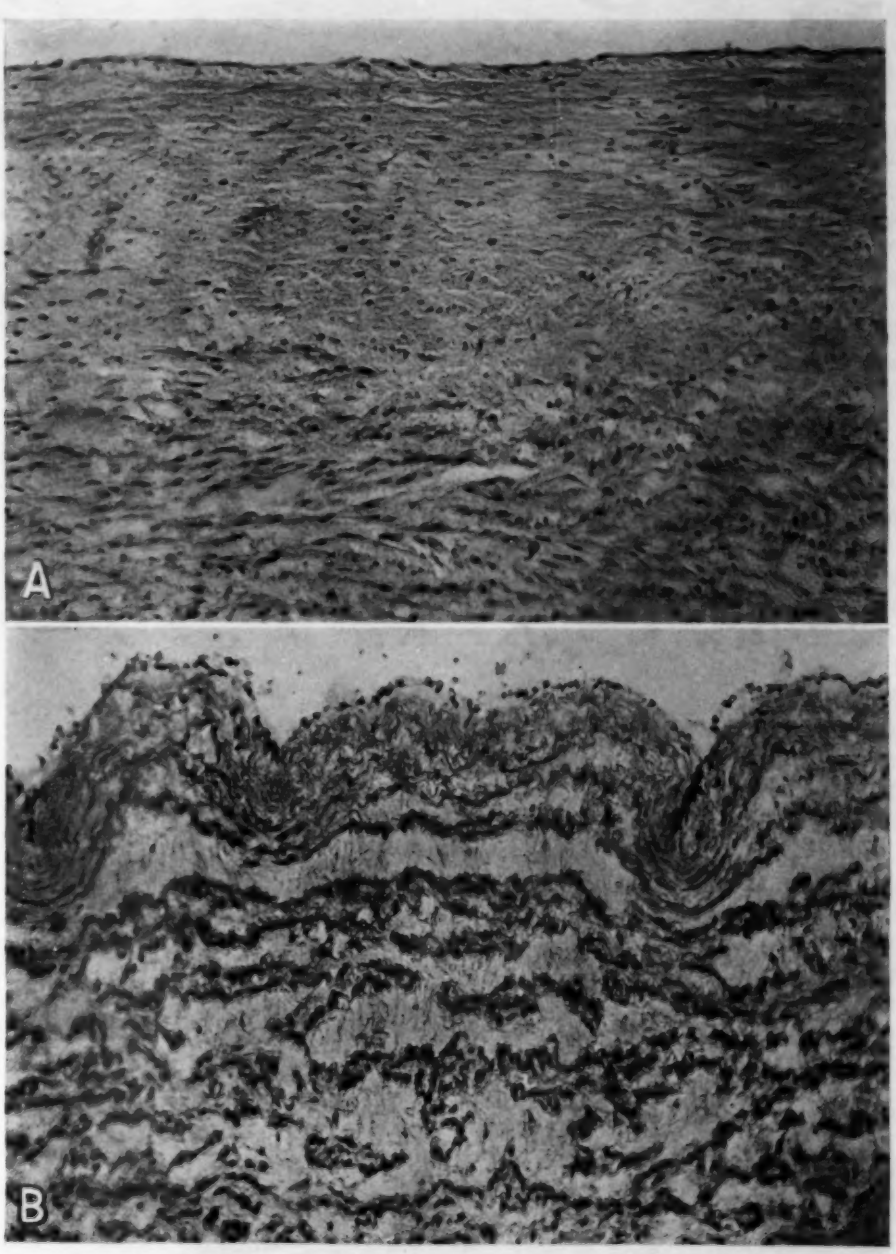

Fig. 1.—*A*, thick intimal fibrohyaline cushion of the ascending aorta in a dog treated with potassium thiocyanate. *B*, loose fibrillar thickening of the intima overlying the highly edematous inner part of the media of the thoracic aorta of a dog.

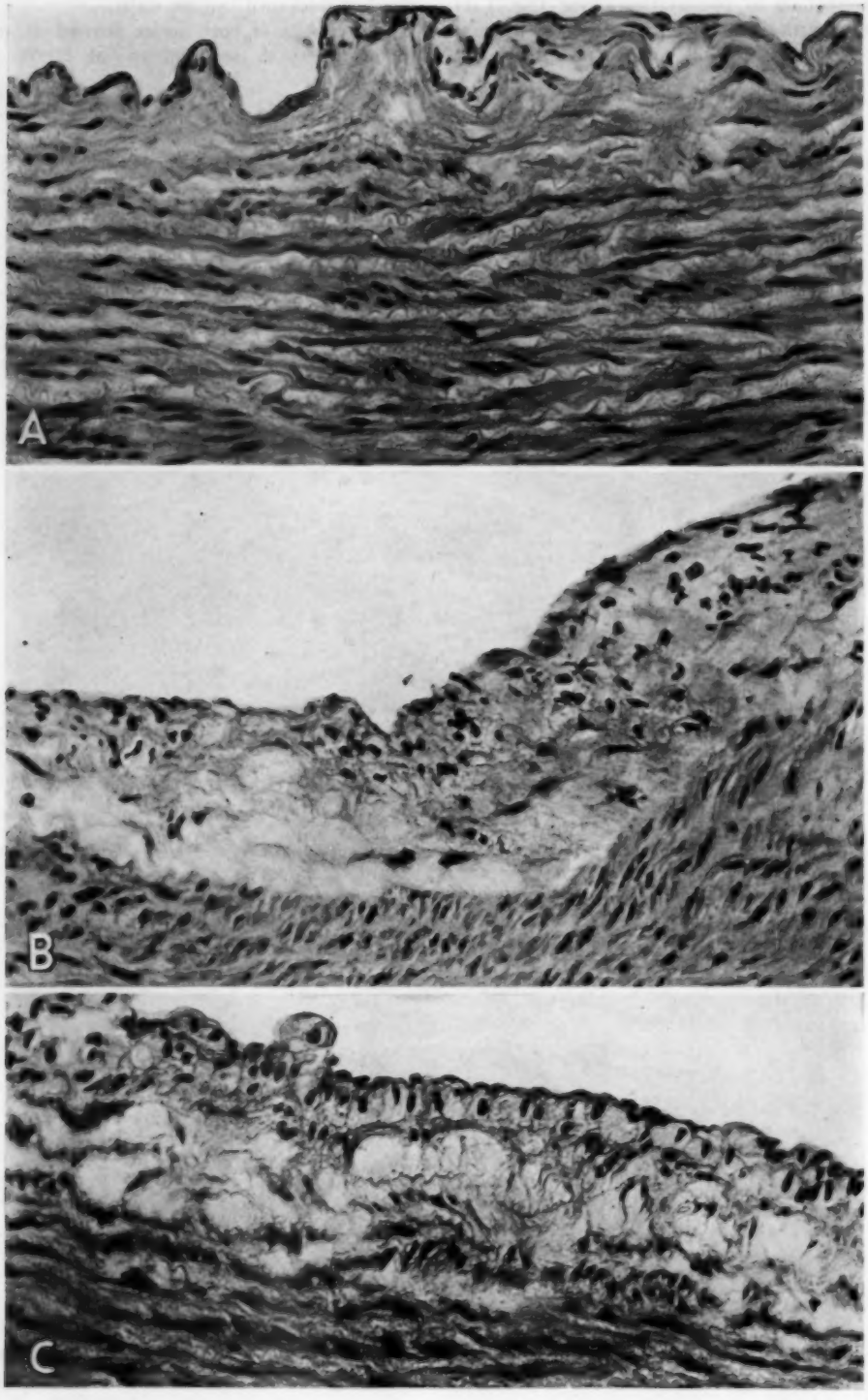

Fig. 2.—*A*, small hyaline thickening of the intima of a small branch of the aorta of a dog. *B*, foam cell cushion of the ascending aorta with degeneration of the deeper parts of the cushion. *C*, foam cell endothelial lining of the aorta of a rabbit, with proliferation of the endothelial cells, which in places project like small balloons into the vascular lumen. The subintimal space is edematous.

For the histologic study of the aortic changes this vessel was usually cut into twelve to fifteen rings. With 2 rabbits of each series, the entire aorta was rolled up and longitudinal sections were made in steps through its entire width and length.

The rabbits of the potassium thiocyanate series had thyroid glands composed of large follicles filled with solid pink-stained colloid. All other organs, including the aorta and its large branches (the carotid and iliac arteries), as well as the arteries of the various parenchymatous organs, were normal. The follicular arteries of the spleen of 1 rabbit showed subendothelial hyalinosis.

The thyroid glands of the rabbits of the potassium thiocyanate–cholesterol series were composed of large follicles with solid colloid. In 2 rabbits foam cell intimal thickenings of the ascending aorta were seen; the aortas of the other 3 rabbits of this series were normal. The foam cell cushions consisted in general of only one to two layers of cells, which were evidently transformed endothelial cells or their derivatives (fig. 2 *B*). Beneath one of these cushions an acellular mucinous mass was noted, representing apparently degenerated foam cells (fig. 2 *C*). In 2 rabbits the follicular arteries of the spleen showed marked subendothelial hyalinosis. The other organs were normal. There was particularly no evidence of an organic lipoidosis in the lungs, the liver, the spleen or the kidneys.

It is noteworthy that the aortic foam cell lesions were found in the 2 rabbits which were killed two weeks after the arrest of the administration of cholesterol; the 3 rabbits without any vascular changes were killed eight weeks after this date.

In 5 control rabbits which received cholesterol by mouth only, there were extensive and marked foam cell cushions in the intima of the aorta and of the large branches.

COMMENT

The observations made lend support to the report of Malisoff,[1] who noted that rabbits treated with cholesterol and a thiocyanate showed reduced development of atheromatous aortic lesions. In view of the small number of animals employed in the present experiment it is doubtful whether or not the thiocyanate treatment continued for eight weeks after the cessation of the cholesterol feeding aided in the removal of lipoids from the aortic intima or whether it entirely prevented the deposition of this material.

Thiocyanate medication apparently did not influence noticeably the increase of serum cholesterol in the rabbits fed cholesterol, but it elevated the cholesterol level of the blood of the dogs as well as of the rabbits which were kept on a normal diet into a normal high or a definitely abnormal high range. While Lindberg, Wald and Baker [2] found in dogs after the administration of toxic doses of potassium thiocyanate a reduction in plasma cholesterol, Westphal and Blum [3] reported that patients treated with thiocyanates exhibited increased blood cholesterol values. This observation is in agreement with the fact that prolonged administration of thiocyanate causes in man and animals goitrogenic hyperplasia of the thyroid gland associated with hypothyroidism or even myxedematous symptoms (Rawson, Hertz and Means [4]; Forster [5]; Barker and Davis [6]; Marine, Rosen and Cipra [7]; Marine, Baumann and Cipra [8]). It is thus evident that thiocyanates prevent the deposition of lipoids in the aortic intima not by lowering the blood cholesterol level or by increasing the production of thyroid hormone but probably by exerting a stabilizing influence on the solution of the colloidally dispersed cholesterol. This action is suggested by the fact that thiocyanates do not penetrate into cells but remain extracellular, where they are colloidal antagonists of cholesterol.

While Taubmann, as well as Wald, Lindberg and Barker,[9] noted the development of anemia in patients subjected to prolonged and sometimes toxic treatment with thiocyanates, with the blood thiocyanate level reaching or surpassing values of 8 to 9 mg. per hundred cubic centimeters, the dogs and the rabbits in which the blood thiocyanate level was kept between 4 and 6 mg. per hundred cubic centimeters exhibited a definite and progressive increase in the amount of hemoglobin in most instances. This difference in the reaction of the blood to thiocyanates is attributable to differences in the doses of thiocyanates administered to man and animals. Thiocyanates thus possess properties similar to iodides, which, like thiocyanates, elicit cutaneous rashes and prevent the development of cholesterol atheromatosis in rabbits, and to nitrites which, in agreement with thiocyanates, lower the blood pressure and may cause anemia or hyperhemoglobinemia and

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3. Westphal, K., and Blum, R.: München. med. Wchnschr. **73**:1187, 1926.

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vascular degenerations (Hueper and Landsberg [10]). The albuminous, fibrotic and hyaline intimal thickenings in the aorta, its branches and the splenic arterioles are attributable to the hypotensive effect of thiocyanate and resemble the arterial lesions seen in animals and in man subjected to chronic nitrite poisoning.

SUMMARY

Potassium thiocyanate administered to dogs and rabbits kept on a normal diet produces a moderate increase of the hemoglobin content and of the cholesterol level of the blood and causes development of albuminous, fibrotic and hyaline intimal lesions in the aorta, its branches and the splenic arterioles.

Rabbits fed cholesterol and potassium thiocyanate show hypercholesteremia and hyperhemoglobinemia, but the production of atheromatous lesions in these animals is distinctly impaired.

The preventive action of thiocyanate on the development of cholesterol atheromatosis is not due to a lowering of the blood cholesterol level or to an increase in the production of thyroid hormone but is probably related to a stabilizing effect on the colloidal equilibrium of the plasma.

10. Hueper, W. C., and Landsberg, J. W.: Arch. Path. **29**:633, 1940.

CORRELATION OF THE ALTERATIONS IN MAMMARY GLANDS, PITUITARY BODY AND OVARIES OF PARABIOTIC RATS

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PHILADELPHIA

In the various experiments reported in the literature on the effects of estrogen on the mammary glands, estrogens have been used in large doses. This has meant intermittent effects when estrogen was injected, or if continuous effects were obtained by implantation of pellets, the doses might be greater than could be secreted by living ovaries.

The method of parabiosis has been used in the present experiments as a means of studying the effect of naturally secreted estrogen in a dosage that can be produced by an animal's ovaries. It is well known that when a gonadectomized animal is joined in parabiosis with an intact female, the follicle-stimulating hormone from the pituitary gland of the gonadectomized rat, which is secreted in excess, passes over to the partner and stimulates the follicles to secrete estrogen. After an interval, continuous estrus is maintained in the recipient without the development of antihormones or any resistance to the effect of the follicle-stimulating hormone (Du Shane and associates [1]). Estrogen does not pass back to the donor rat, which continues to show absence of estrus, atrophied mammary glands and castration cells in the pituitary gland.

This method has proved to be a useful one for studying the effect of endogenous estrogen on the mammary glands and is a method which apparently has not been used previously for this purpose. The changes occurring in the mammary glands have been reported briefly (Zeckwer [2]). In the course of continuing these experiments it became evident that changes were occurring in the stimulated ovaries which merit description. In previous experiments reported in the literature on the effect of estrogen, the interest was directed toward changes in the mammary glands, in the pituitary body or in the ovaries, and little was done to relate the changes in one with the changes in the others. The present experiments are presented as an attempt to do this.

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METHODS

Two rats of the same age and usually of the same litter were anesthetized with ether and then united by suturing together their abdominal walls and skin. Usually at the same time, occasionally at a later time, one rat (for convenience called the donor) was gonadectomized. Various combinations have been studied, an ovariectomized female united to an intact female, a castrated male united to an intact male, a castrated male united to an intact female. The experiments described in this paper deal chiefly with the first category. Atrophic changes in the gonadectomized donor are irrelevant. Changes subsequently described are those observed in the recipient parabiont. The control in each instance was a single intact litter mate or two litter mates united but neither one gonadectomized, or occasionally a single rat of the same age but not a litter mate.

Two separate colonies of rats were used. One consisted of descendants of a single pair of rats from Wistar Institute which had been maintained in this laboratory since 1941; the other was of mixed origin but inbred in this laboratory since 1936. In these rats corpora lutea first appeared between the ages of about 55 and 60 days. Some of the rats were operated on before puberty; others, after they were 60 days of age.

Vaginal smears were made when this was indicated. At intervals biopsies were made of mammary glands. At the termination of the experiment complete autopsies were usually performed. Occasionally torsion of the pedicle uniting the rats developed, and with the spontaneous obliteration of the circulation the rats became separated without harm, maintaining a separate existence. In these rats the recovery of the mammary glands, ovaries and pituitary body was studied. In other pairs in which the pedicle became narrow, trypan blue was injected in one in order to note the appearance of the dye in the partner as evidence that the circulation between them was intact.

In a few pairs, the uterus of the recipient, in others the thyroid gland and in a few the adrenal glands of the recipient, were removed. These operations were followed by no appreciable modification of the response of ovaries and the mammary glands.

Studies were made of 75 estrogenized rats and 70 controls. Mammary glands were studied in 60 estrogenized rats (42 autopsies, 59 biopsies) and in 36 controls at autopsy. Ovaries were studied in 56 estrogenized rats and 65 controls. Additional controls were provided in rats used for breeding and rats used for a variety of experiments, autopsies of which indicated that spontaneous lesions of mammary glands and other endocrine organs were rare in the two colonies.

RESULTS

Females.—The results are expressed in the accompanying table. The changes observed can be roughly divided into four stages: In the first or early stage after operation, lasting about one week, the principal effect of the follicle-stimulating hormone secreted in excess by the gonadectomized rat's pituitary gland was the stimulation of the recipient's ovarian follicles, which enlarged (figs. 1 and 2). The mammary glands showed little change if any at this stage. The pituitary body showed some loss of granules from basophils and acidophils.

In the second period, beginning at the second week, the enlarged follicles in young rats were transformed to large and numerous corpora lutea (fig. 4) although in litter mate single controls of these rats corpora lutea had not yet developed (fig. 3), and the corpora lutea in older rats were larger than those in the controls. Presumably, the recipient's pituitary gland was lost because the lutein cells that had not yet disintegrated appeared scattered in small groups (fig. 17). Polymorphonuclear leukocytes and mononuclear phagocytes occurred in areas where the lutein cells were breaking up. The estrogen secreted by the stimulated ovarian follicles had caused degranulation of the chromophil cells of the pituitary gland, and this was associated with failure of secretion of luteinizing hormone (the evidence for which will be reviewed later), so the follicles of the ovary did not go on to luteinization. The alveolar cells of the mammary glands were actively secreting a substance which stained intensely with eosin and which often was grossly visible on cutting through the gland, having the appearance of thick white milk (figs. 11 and 12). The pituitary gland was enlarged and very vascular; it consisted chiefly of nongranular cells.

During the fourth period, beginning at about sixteen weeks, corpora lutea disappeared and the ovaries became composed entirely of follicles, often cystic, and solid masses of vacuolated granulosa cells in the stroma between follicles (figs. 13 and 14). Such solid masses of cells are sometimes called "interstitial cells." This term gives rise to confusion and misunderstanding. There is good evidence that such cells are derived from the theca interna of atretic follicles, and in the rats of the present experiments the arrangements of these cells into groups strongly supported this interpretation. Their large size and vacuolation indicate that they are in a stage of active secretion, and their appearance is identical with cells lining cystic follicles. Numerous widely dilated capillaries in close relation to these cells gave additional evidence that the ovary was stimulated to active secretion. Most of the control rats showed many corpora lutea; the exceptions were 2 old rats (aged 346 and 772 days) with ovaries devoid of corpora lutea. The mammary gland secreted so actively that cystic distentions of alveoli and ducts occurred (fig. 20). The changes progressed in intensification of hyperplasia and secretion. In 6 rats, in localized regions of the mammary gland stroma de-

Changes in Mammary Glands and Ovaries

	Stage 1		Stage 2		Stage 3	Stage 4
Days after operation	7-10	7-10	11-35	11-35	36-112	113-470
Days of age at operation	25-49	63-87	21-39	34-115	22-361	26-792
Days of age at death or biopsy	35-56 prepuberal	70-94	39-57 prepuberal	57-143	74-470	162-792
Mammary glands						
No change	4 (A), 3 (B)	3 (A)	2 (A), 1 (B)	.....	.....	.....
Hyperplasia of duct epithelium with no alveolar hyperplasia	.....	.....	4 (A), 1 (B)	.....	.....	.....
Hyperplasia of alveoli with no secretion	.....	.....	2 (A), 1 (B)	5 (A), 5 (B)	1 (A), 2 (B)	.....
Hyperplasia of alveoli with slight secretion	.....	.....	2 (A)	1 (A), 2 (B)	3 (A), 9 (B)	4 (B)
Hyperplasia with cystic distention of ducts and alveoli due to copious secretion	.....	.....	.....	.....	3 (A), 14 (B)	4 (A), 17 (B)
Tumors	.....	.....	.....	.....	.....	8 (A)
Ovaries of experimental rats						
Enlarged follicles only, without increased luteinization	6	2	2	..	..	..
Increased luteinization	1	2	8	13	..	..
Regressive corpora lutea	..	..	..	..	4	2
Absence of corpora lutea, ovary composed entirely of granulosa cells	..	..	..	..	4	12
Ovaries of control rats						
Normal immature ovary without corpora lutea	3	..	9	..	..	..
Normal mature follicles and corpora lutea	..	4	..	11	12	24
Absence of corpora lutea	..	..	..	..	..	2

A = autopsies; B = biopsies.

had released luteinizing hormone, the evidence for which will be discussed later. At this time the mammary glands of prepuberal rats showed proliferation of epithelium in the ducts (figs. 5 and 6) and those of some rats, both young and old, showed for the first time hyperplasia of the acini forming lobules (figs. 7 and 8), a change ascribed to the effect of progestin secreted by the corpora lutea. The acini of the mammary glands showed no secretion at this time in most of the rats. Histologically, the pituitary gland showed loss of granules, as previously reported (Zeckwer [3]).

During the third period, beginning about five weeks after operation, cystic follicles were numerous, and corpora lutea were regressing or had completely disappeared (figs. 9 and 10). First there was shrinkage of lutein cells. Those in the center disappeared, and their places were taken by vacuoles of presumably fatty material within phagocytes. A yellow-brown pigment was scattered in close relation to these vacuoles. The definition of the periphery of the corpus luteum

3. Zeckwer, I. T.: Arch. Path. **30**:461, 1940.

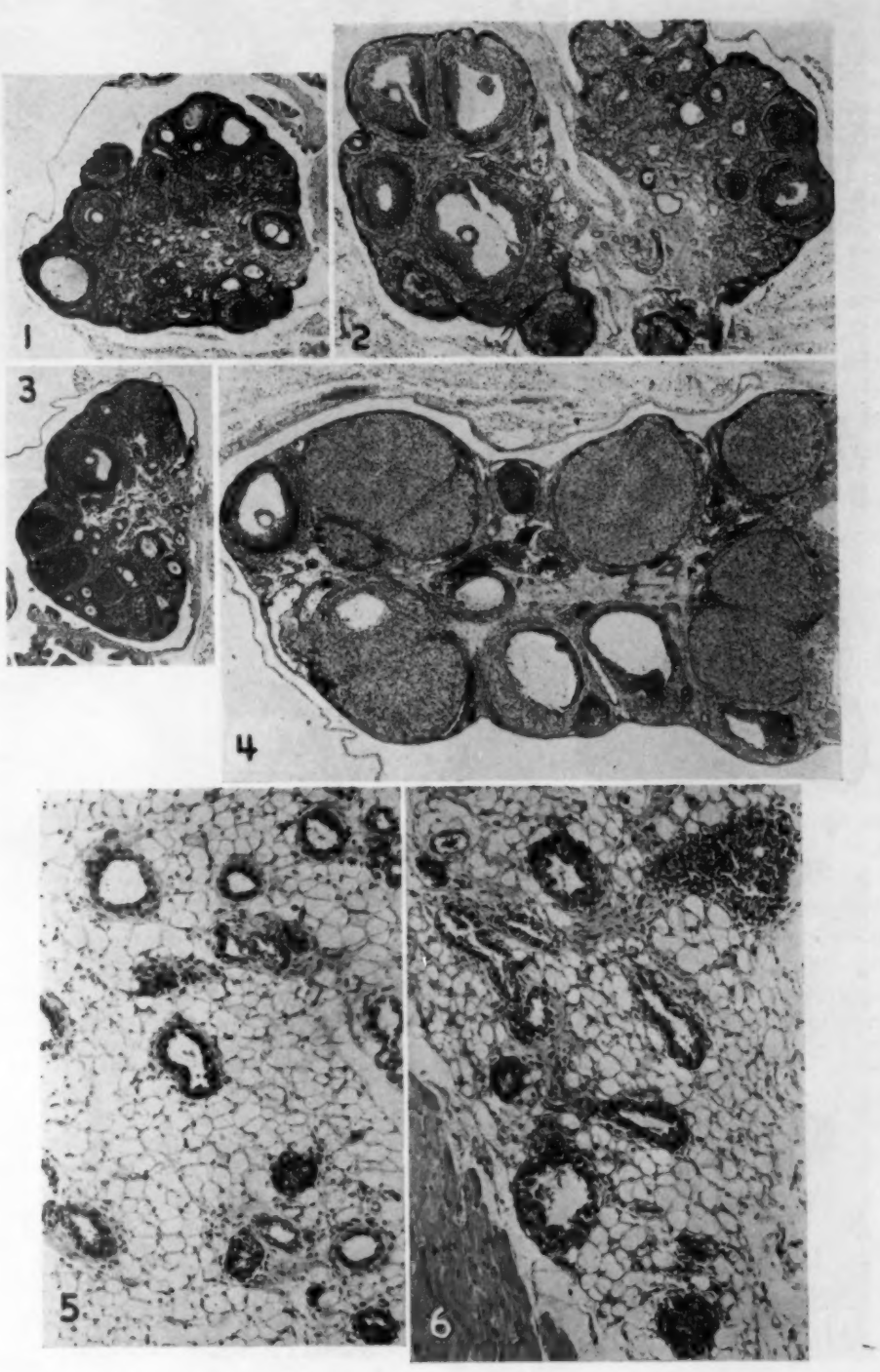

Fig. 1.—Ovary of rat 26-W-8, a normal control immature rat, aged 46 days; × 25. No corpora lutea.

Fig. 2.—Ovary of rat 26-W-9, a litter mate of rat 26-W-8, aged 46 days, seven days after parabiosis; × 25. Follicle ripening. No corpora lutea.

Fig. 3.—Ovary of rat 27-C-6, a normal control immature rat, aged 35 days; × 25. No corpora lutea.

Fig. 4.—Ovary of rat 27-C-4, a litter mate of rat 27-C-6, aged 35 days, ten days after parabiosis; × 25. Many large corpora lutea.

Fig. 5.—Mammary gland of normal immature rat 27-C-6, aged 35 days; × 72.

Fig. 6.—Mammary gland of 27-C-4, aged 35 days, ten days after parabiosis; × 72. Little change in mammary glands except slight thickening of ducts.

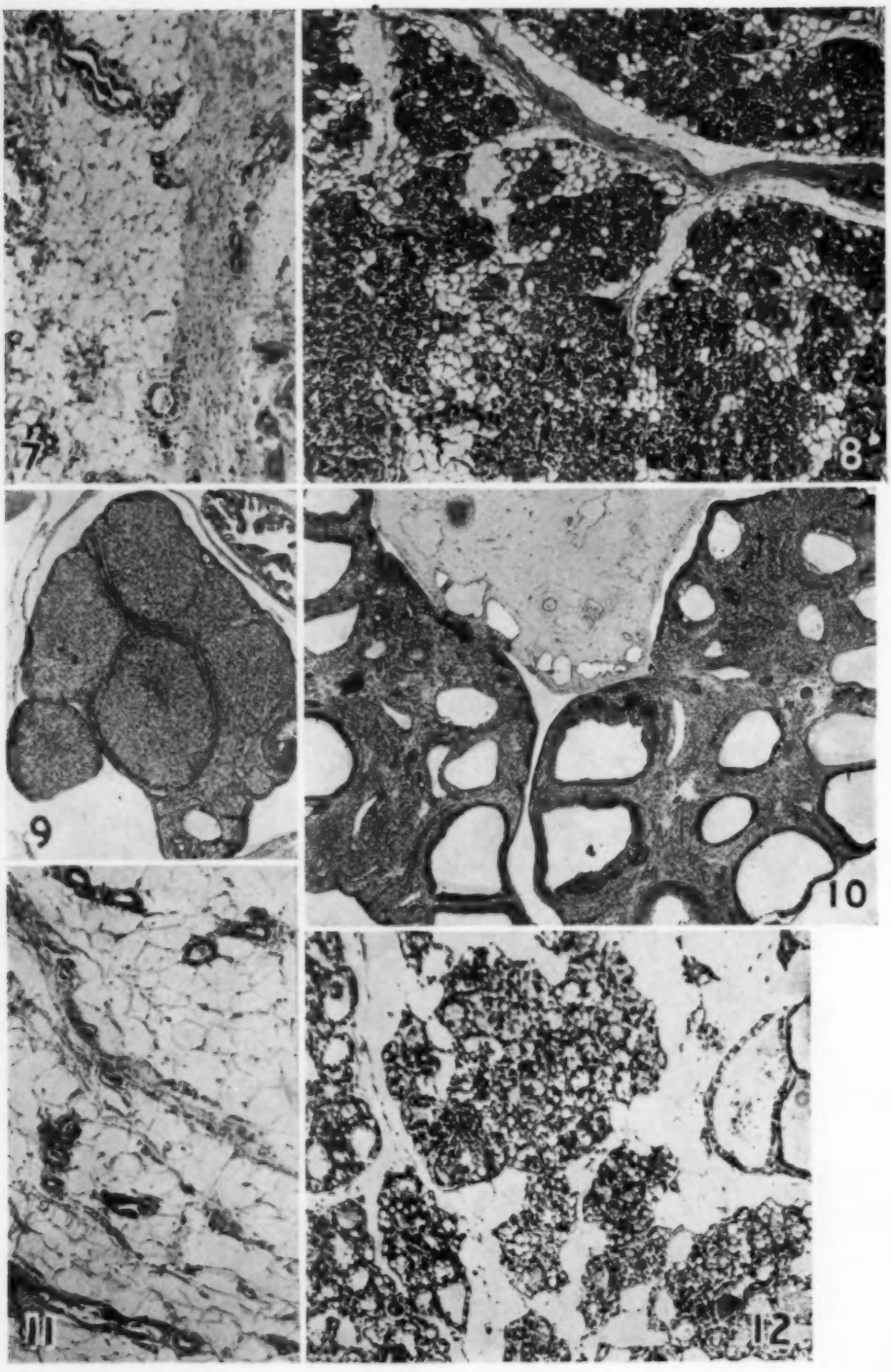

Fig. 7.—Mammary gland of normal mature rat 18-F-5, aged 79 days; × 62.

Fig. 8.—Mammary gland of rat 18-S-9, aged 62 days, twenty days after parabiosis; × 62. Extensive hyperplasia of alveoli without secretion.

Fig. 9.—Ovary of normal rat 21-BC-6, aged 162 days; × 25. Corpora lutea.

Fig. 10.—Ovary of rat 21-BC-4, 162 days of age, one hundred and nineteen days after parabiosis; × 25. Cystic follicles (corpora lutea have regressed, except for a few irregular clusters of lutein cells).

Fig. 11.—Mammary gland of normal rat 21-BC-6, aged 162 days; × 72.

Fig. 12.—Mammary gland of rat 21-BC-4, aged 162 days, one hundred and nineteen days after parabiosis; × 72. Hyperplasia of alveoli with secretion. Cystic distention of some acini.

veloped and a circumscribed nodule of microscopic size was formed, composed of secreting alveoli with supporting fibrous tissue (fig. 18). Because these areas were circumscribed and distinct from the diffuse glandular hyperplasia elsewhere in the gland, it was considered justifiable to designate them as fibroadenoma, even though they were of minute size. In 1 rat such a growth developed into a large projecting tumor, 5 cm. in diameter (figs. 15 and 16).

Two rats showed a different type of adenoma, consisting of very cellular areas, solidly packed with cells, with a lumen only occasionally visible and with a scarcely recognizable glandular pattern (figs. 19 and 20). This type was first noticed at 162, 169, 203, 235, 373, 415, 437 and 449 days of age. No cancer of the mammary gland occurred.

Litter mate control rats showed no spontaneous tumors, but among the rats used for breeding 3 were found to have spontaneous fibroadenoma, first observed at 232, 332 and 618 days of age, the tumors attaining sizes of 1.5 cm. and 2 cm. in diameter. One of these had much milk secretion. In another rat a tumor developed during pregnancy, first noticed at 138 days of age, very cellular and nonsecreting at biopsy, which regressed and at the time of autopsy, at 549 days of age, had disappeared completely. These were the only gross spontaneous tumors of mammary glands observed.

In late stages the pituitary body became enormous, expanding as a large mass at the base of the brain. It was very vascular and soft and was composed chiefly of chromophobe cells, with only an occasional small acidophil seen. These changes were identical with those described as resulting from long-continued dosage with exogenous estrogen, to be discussed later. There was stunting of skeletal and visceral growth, presumably due to lessened secretion of growth factor by acidophils. The skin became thin and delicate, and the hair became finer in texture. In those rats whose blood sugar was determined there was a lowered level of blood sugar, which may mean decreased production of the diabetogenic factor of the pituitary gland, with unopposed secretion of insulin. Eventually the rats became cachectic and died for no obvious reason; it seems reasonable to ascribe this decline to pituitary failure and possibly to the effects of pressure at the base of the brain.

Males.—Seven male rats were united with castrated males. Sections of the mammary glands were obtained at intervals varying from forty-two to three hundred and eighty-five days after operation (5 autopsies, 7 biopsies). They showed considerable hyperplasia with secretion, but less distention of the lumens than did sections of the mammary glands of females, as illustrated (figs. 21, 22 and 23). There was no stunting of body growth and relatively little change in the pituitary body.

Recovery After Separation.—Eight female rats were studied after they had become separated. Three rats that had been united for one hundred and eleven to one hundred and twenty-four days and separated from sixteen to fifty or more days showed regression of the mammary glands as indicated by flat inactive epithelium and fewer alveoli, but secretion still remained in cystic distentions of ducts and acini. The ovaries had recovered, as indicated by the presence of corpora lutea. The other 5 rats showed regression of the mammary glands but their ovaries had not recovered. In 3 rats that had been united for three hundred and fifty-seven, four hundred and thirty-one, and eighty-six days and had been separated for thirty, sixty-one, and two hundred and fifty-one days respectively, the ovaries were composed entirely of granulosa cells with no corpora lutea. The other 2, united for fifty-three and one hundred days and separated for two hundred and three and three hundred and seventy-four days, showed predominance of granulosa cells with only occasional small corpora lutea. This failure to luteinize even after long periods of separation shows that the pituitary gland had not regained its power to secrete luteinizing hormone; the pituitary gland remained large, vascular and soft, with decreased numbers of chromophil granules. Estrogen can therefore produce changes in the pituitary gland that are irreversible. Probably old age was a factor.

COMMENT

For reviews of the literature on the effect of estrogen, progestin and pituitary factors on the mammary glands, the reader is referred to Turner,[4] Riddle [5] and Geschickter.[6]

In the first stage in immature rats, the secretion of estrogen by the stimulated ovaries of some rats is uncomplicated by any development of corpora lutea. Although estrogen probably stimulates the pituitary gland to secrete a factor (mammogen I) which promotes duct growth in the mammary gland (Gomez and Turner [7]; Mixner and Turner [8]; Leonard and Reece [9]; Greep and Stavely [10]), no effect was noted in most of the parabiotic rats examined in this stage.

In the second stage, in which estrogen has acted for a longer time, the development of corpora lutea in young rats and the enlargement of those in older rats are consistent with the evidence reviewed by Fevold [11] and by Allen, Hisaw and Gardner [12] that estrogen stimulates the pituitary gland to release luteinizing hormone. Apparently this effect of estrogen is dependent on the interval of time and the size of dose. Witschi and Levine [13] observed that when a female rat was

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12. Allen, E.; Hisaw, F. L., and Gardner, W. U., in Allen, E.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 452.

13. Witschi, E., and Levine, W. T.: Proc. Soc. Exper. Biol. & Med. **32**:101, 1934.

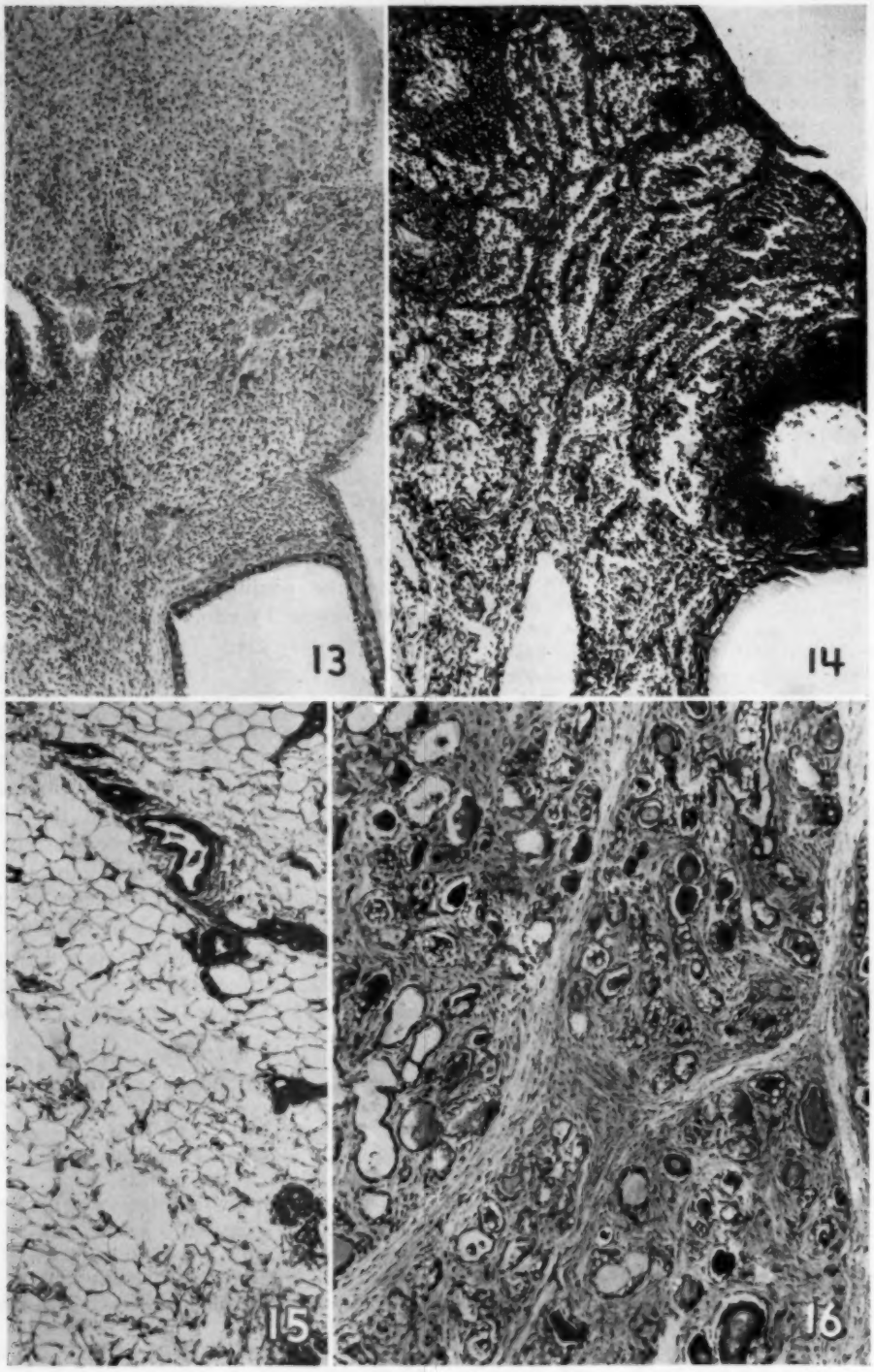

Fig. 13.—Ovary of control rat 18-I-7, aged 433 days; × 62. Corpora lutea.

Fig. 14.—Ovary of rat 18-I-6, aged 433 days, four hundred and seven days after parabiosis; × 62. No corpora lutea; ovary entirely composed of vacuolated granulosa cells.

Fig. 15.—Mammary gland of normal rat 18-I-7, aged 433 days; × 62.

Fig. 16.—Mammary gland of rat 18-I-6, aged 433 days, four hundred and seven days after parabiosis; × 62. Lactating fibroadenoma.

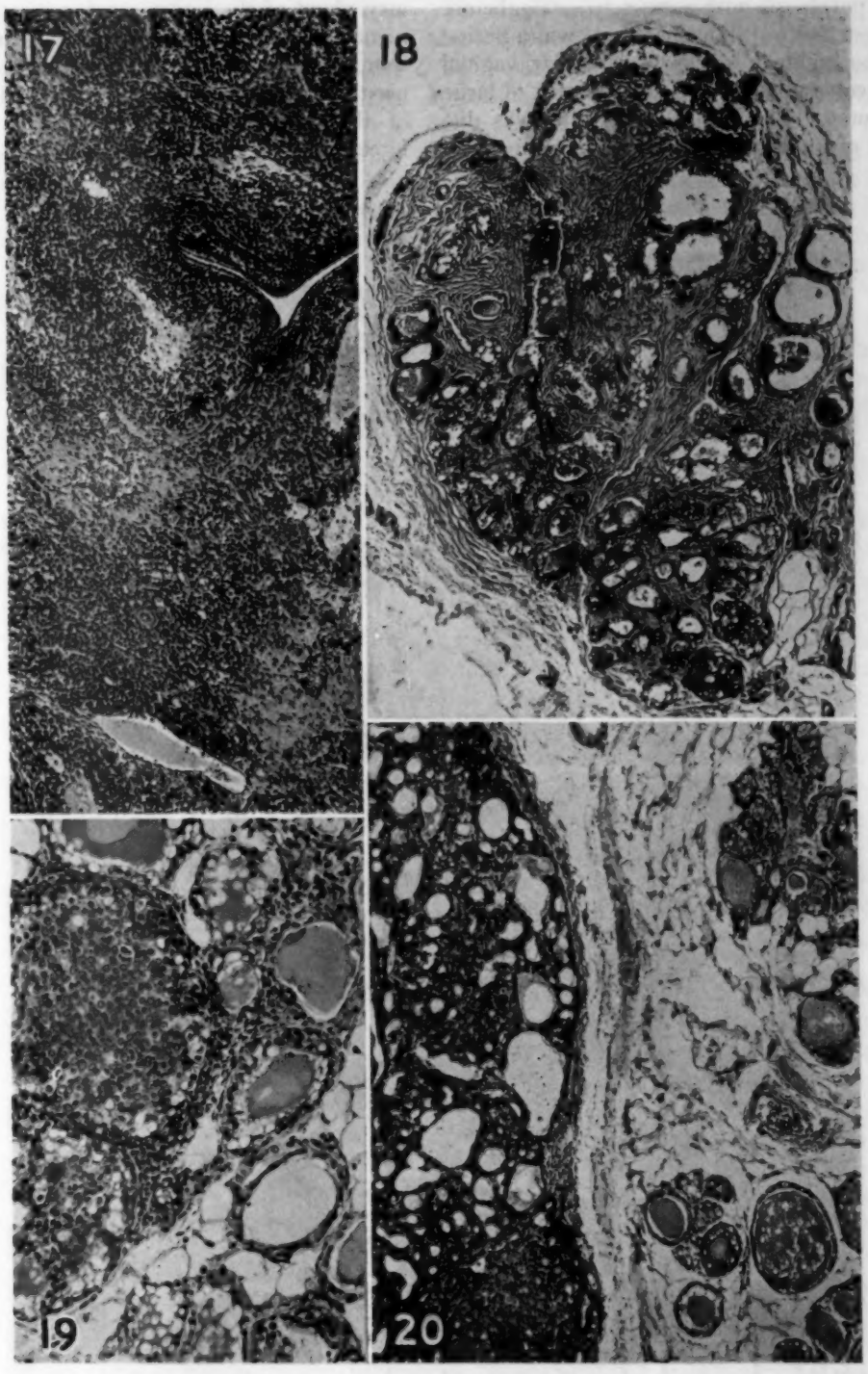

Fig. 17.—Ovary of rat 20-T-5, aged 169 days, one hundred and twenty days after parabiosis; × 72. Ovary composed almost entirely of granulosa cells, with only a few degenerating lutein cells remaining.

Fig. 18.—Mammary glands of preceding rat, 20-T-5, 169 days of age, one hundred and twenty days after parabiosis; × 72. Fibroadenoma.

Fig. 19.—Mammary gland of 16-J-6, 235 days of age, one hundred and eighty-nine days after parabiosis; × 165. Adenoma. Distention of acini with milk secretion.

Fig. 20.—Mammary gland of 15-V-8, 415 days of age, three hundred and sixty-seven days after operation; × 62. Adenoma. Distention of acini with milk secretion.

united in parabiosis with a cas rated male, before constant estrus was established there was a period of three to eighteen weeks of irregular vaginal smears accompanied by the development of large corpora lutea. They proved that this was due to release of luteinizing hormone from the pituitary gland of the intact rat, for in other experiments in which the female rat was hypophysectomized and a sufficient length of time elapsed to permit atrophy of preexisting corpoa lutea, union of this rat with a castrated male caused no luteinization and permitted immediate establish-

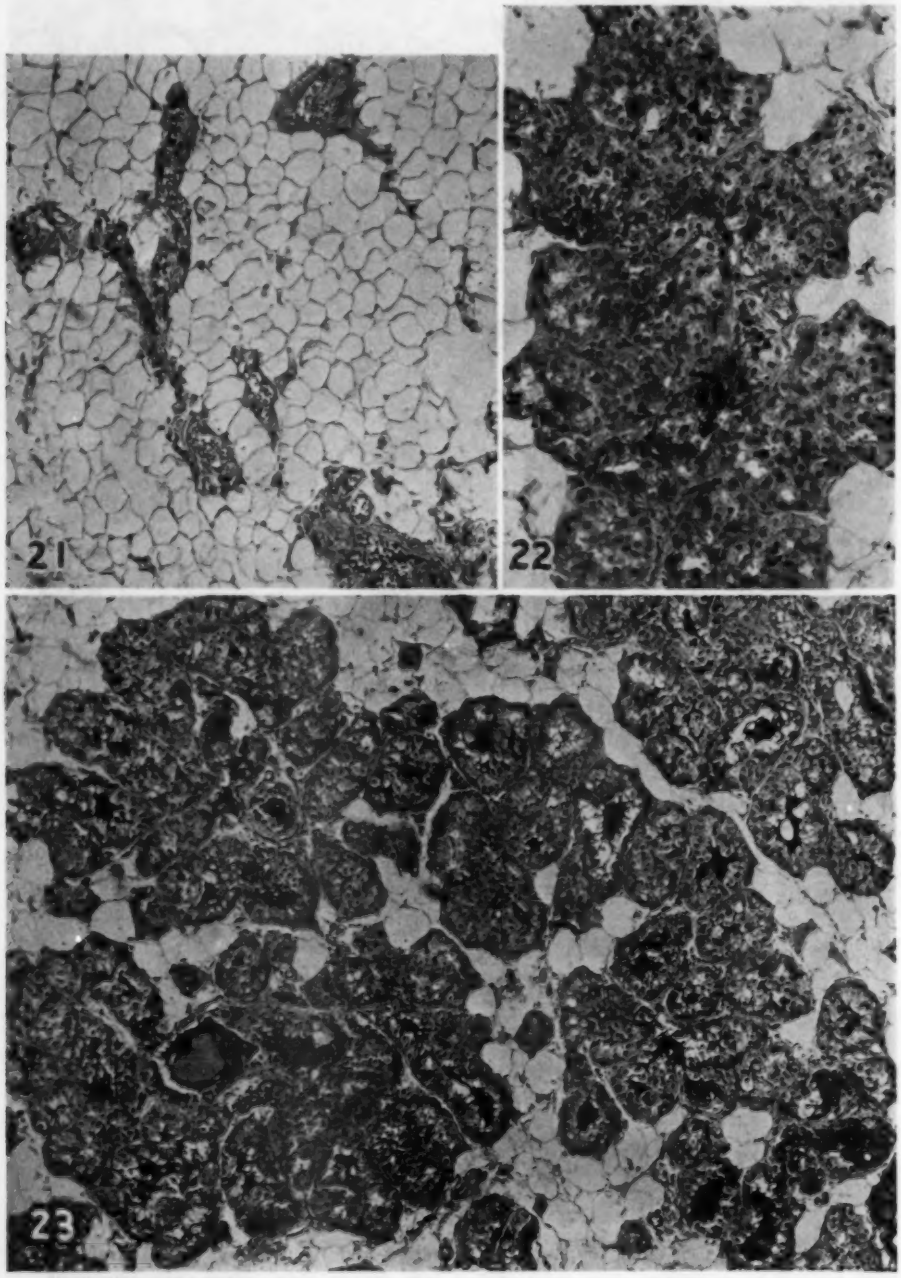

Fig. 21.—Mammary gland of male rat 18-F-2, 403 days of age, three hundred and sixty-six days after castration, united with rat 18-F-1; ×72. Atrophy of castration.

Fig. 22.—Mammary gland of male rat 16-M-1, aged 396 days, three hundred and forty-eight days after parabiosis; ×165. Hyperplasia of acini.

Fig. 23.—Mammary gland of male rat 18-F-1, 403 days of age, three hundred and sixty-six days after parabiosis with 18-F-2; ×72. Hyperplasia of acini with secretion.

ment of constant estrus. In these experiments they did not study the mammary glands.

Merckel and Nelson [14] observed that repeated doses of estrogen given to adult rats maintained corpora lutea for about three weeks but that single or repeated large doses given to immature rats did not cause formation of corpora lutea. The latter observation seems difficult to understand and differs from the results of other workers. In the adult rats estrone caused proliferation of ducts and alveoli of mammary glands; in the immature rats the alveolar system was not developed but ducts and end buds were present.

The hyperplasia of the alveoli of the mammary gland in stage 2 may be attributed to the additional factor of progestin secreted by the corpora lutea acting on the pituitary gland to cause release of a factor promoting lobular alveolar growth, presumably mammogen II, which is consistent with the observations of Gomez and Turner,[7] Mixner, Bergman and Turner,[15] Mixner and Turner [8] and Nelson.[16]

One may compare the hyperplasia of the mammary gland without secretion in the parabiotic rats when corpora lutea are large with the develment of the mammary gland in women during pregnancy when progestin formation predominates in the maintenance of pregnancy. Consistent with this is the observation that in pseudopregnant rabbits, with the presence of corpora lutea, the mammary gland becomes well developed but no lactation occurs. The lactogen content of the pituitary gland remains low in such animals (Meites and Turner [17]).

However, in 4 young rats with very large corpora lutea, only duct growth had occurred, which raises the question of whether secretion of progestin is really the determining factor in alveolar development, rather than the duration of the estrogenic effect.

In stage 3, as secretion of estrogen continues, corpora lutea regress and disintegrate. This can be attributed to failure of formation and release of luteinizing factor, which may be due to estrogen inhibiting the production and release of this factor or due to the pituitary gland having been stimulated to release luteinizing factor to the point that the gland has become exhausted. Hill [18] noted that estrus became constant in parabiotic rats when corpora lutea disappeared between sixty-six and one hundred and thirty days. If the gonadectomized rat was a male instead of a female, the regression of corpora lutea was more rapid, because the potency of the male pituitary gland is greater. Witschi and Levine [18] noted in parabiotic rats that a constant and high level of estrogen suppressed luteinizing hormone. Likewise DuShane and associates [1] concluded that the release of luteinizing hormone became gradually suppressed.

Daily injections of estrogens result in disappearance of corpora lutea at forty-nine, sixty-four and ninety days, according to Morrell and Hart,[19] while Nelson [20] obtained regression of corpora lutea after twenty days of injection of estrogen, which Merckel and Nelson [14] attributed to exhaustion of the pituitary gland in its supply of luteinizing hormone, as evidenced by degranulation of the chromophil cells of the gland.

Usually concomitant with the regression of corpora lutea, milk was secreted into the acini of the mammary glands of the parabiotic rats to the point of forming milk cysts. In the past there has been much debate about the factors which cause lactation. Some have expressed the view that lactation is suppressed by large amounts of estrogen. Experiments, reviewed by Nelson,[16] supported this interpretation, and many obstetricians use estrogen to suppress lactation post partum. This view is still widely held in spite of the accumulation of much evidence against it, which has been carefully reviewed in a series of studies on lactation by Meites and Turner [17] and in a recent article by Novak,[21] to which the reader is referred. Briefly stated, some of the evidence against estrogen inhibiting lactation is as follows: 1. The increase of weight in suckling rats is not a valid measure of lactation, as estrogen is excreted in the milk and after being absorbed by the young inhibits their growth (Weichert and Kerrigan [22]). 2. Post partum the child is removed from the breast at the time that estrogen is given to the mother. It is well established that the mechanical and nervous factors involved in suckling maintain lactation and that if the child continues to nurse at the breast, estrogen given to the mother does not inhibit lactation. 3. Assay of lactogenic hormone in the pituitary glands of mother rats indicates that the level is not lowered by injections of estrogen (Meites and Turner [17])

14. Merckel, C., and Nelson, W. O.: Anat. Rec. **76**:391, 1940.

15. Mixner, J. P.; Bergman, A. J., and Turner, C. W.: Endocrinology **31**:461, 1942.

16. Nelson, W. O.: Physiol. Rev. **16**:488, 1936.

17. Meites, J., and Turner, C. W.: Endocrinology **30**:711, 719 and 726, 1942; **31**:340, 1942; Proc. Soc. Exper. Biol. & Med. **49**:193, 1942.

18. Hill, R. T.: Endocrinology **17**:414, 1933.

19. Morrell, J. A., and Hart, G. W.: Endocrinology **29**:796, 1941.

20. Nelson, W. O.: Anat. Rec. (supp.) **64**:52, 1935.

21. Novak, E.: J. Clin. Endocrinol. **3**:274, 1943.

22. Weichert, O. K., and Kerrigan, S.: Endocrinology **30**:741, 1942.

but is sharply reduced by removing the suckling young when no injection of estrogen is given.

Quite opposed to the view that estrogen inhibits lactation is excellent evidence that estrogen stimulates lactation. Abundant lactation has been produced by injections of estrogen in unmated, sterile and castrated animals. Such experiments reported in the literature have been recently reviewed by Reece.[23] Lactation resulting from the administration of estrogen is due to the increased storage and secretion of lactogen by the pituitary gland caused by estrogen (Meites and Turner[17]; Lewis and Turner[24]).

The present experiments lend support to the view that estrogen stimulates lactation. The secretion of milk in the parabiotic rats, usually after the regression of corpora lutea, may be compared to the low level of progestin in women post partum, which apparently is the condition which releases lactogenic hormone from the pituitary gland. Progestin is known to fall just before delivery, as indicated by decrease in pregnanediol. The corpus luteum of pregnancy has atrophied as the placenta has taken over the formation of progestin, which is known to be dominant during pregnancy. The expulsion of the placenta has caused a sudden reduction in progestin. In this connection the recent observations of Meites and Turner[17] are of much interest. They reported that injections of progestin in suitable doses prevented the effect of estrogen in raising the lactogenic hormone content of the pituitary gland. They stated that progestin does not inhibit the pituitary gland from producing lactogen, but that progestin "overrides the effects of estrogen on lactogen secretion." They expressed the belief that lactation is suppressed during pregnancy because the large amounts of progestin present in pregnancy inhibit the action of estrogen on the pituitary gland and thus prevent stimulation of the secretion of lactogen. The reader is referred to their articles[17] for a review of the literature. In this connection should be mentioned the experiments of Laqueur,[25] who noted that the hyperplasia of the mammary glands induced in virgin female rats by injections of testosterone propionate was followed by lactation only when the corpora lutea regressed.

Although usually the parabiotic rats showed secretion only after regression of corpora lutea had begun, there were 3 rats in which slight secretion occurred in the presence of large corpora lutea in stage 2. Furthermore, in the spontaneous lactating adenofibroma occurring in a single intact rat corpora lutea were present. This suggests that the long-continued effect of estrogen and its predominance over that of progestin may be important in determining lactation.

During stage 4 in the parabiotic rats, the stunting of body growth, the thinning of hair and the enlargement of the pituitary gland, which is composed largely of chromophobe cells, are similar to the effects of long-continued injections of estrogen (Hooker and Pfeiffer[26] and others). Presumably, the stunting of body growth is due to the pituitary gland failing to produce adequate growth hormone.

There is, then, evidence that estrogen causes decreased function of the pituitary body as regards secretion of gonadotropin and growth factor, and this hypofunction has always been difficult to reconcile with the fact that after long-continued injections of estrogen the pituitary gland becomes large and vascular, with hypertrophy of the Golgi apparatus and mitochondria, all changes which are associated with increased rather than decreased function (Severinghaus[27]). I have recently suggested (Zeckwer[28]) that these histologic changes should be regarded as related to hypersecretion of the lactogenic hormone by the pituitary gland on the basis of quantitative data on estrogen causing increased storage and secretion of lactogen by the pituitary gland (Meites and Turner[17]; Lewis and Turner[24]) and on the basis of the present experiments, in which secretion by the mammary gland occurred at the time of enlargement of the pituitary gland. If the concept is accepted that hyperplasia of the pituitary gland resulting from estrogen is related to hypersecretion of lactogen, it will remove the obstacles to a satisfactory understanding of the association of a hyperplastic condition of this gland with failure of secretion of gonadotropin and growth factor. This explanation has probably not been thought of in the past because those who have assayed the lactogen content of the pituitary gland have not been greatly concerned with the histologic changes in this gland, and those like Severinghaus[27] and Wolfe and Brown,[29] who have been concerned primarily with the cytologic aspects of the gland, have thought of function in terms of gonadotropin rather than of lactogenic hormone.

23. Reece, R. P.: Proc. Soc. Exper. Biol. & Med. **52**:145, 1943.

24. Lewis, A. A., and Turner, C. W.: Proc. Soc. Exper. Biol. & Med. **48**:439, 1941.

25. Laqueur, G. L.: Endocrinology **32**:81, 1943.

26. Hooker, C. W., and Pfeiffer, C. A.: Endocrinology **32**:69, 1943.

27. Severinghaus, A. E., in Allen, E.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 1045.

28. Zeckwer, I. T.: Science, to be published.

29. Wolfe, J. M., and Brown, A. D.: Endocrinology **31**:467, 1942.

It is not known what cells of the pituitary gland secrete the lactogenic hormone. Schooley and Riddle.[30] ascribed secretion of lactogen to the acidophils in pigeons. In the present experiments, stunting of the body growth, which may be ascribed to decreased numbers of acidophils and consequent lessened production of growth hormone, was not associated with decrease of the production of lactogen by the pituitary gland, as evidenced by abundant secretion in the mammary gland.

It is interesting that pituitary follicle-stimulating hormone passes over to the partner so readily, while estrogen does not pass over into the other animal. Pituitary lactogen also does not pass over, as the mammary glands of the gonadectomized partner remained atrophied.

No cancers developed either spontaneously or experimentally in the two colonies of rats used in the present experiments. Although in mice with the proper genetic background the injection of estrogen readily leads to cancer, this lesion is not commonly produced in rats. For reviews of hyperplasia and tumors of the mammary glands produced in rats, the reader is referred to recent contributions by Geschickter [6] and Emge.[31]

Witschi and Levine [13] studied parabiotic rats after separation. They stated "the hypophysis of the female in constant estrus retains the potency of producing luteinizer. For such females, if separated from the castrate twins, resume cyclical changes and may become pregnant and have litters again." It was therefore a surprising observation that in 5 of the separated rats of the present series the ovaries and the pituitary gland did not recover. Morrell and Hart [19] also observed recovery when repeated injections of estrogen causing disappearance of corpora lutea were stopped. After twenty-three days recovery of the ovaries occurred. Age may be the factor.

In male rats, hyperplasia of the mammary gland has been produced by injections of testosterone propionate (Laqueur [25] and others), and Reece and Mixner [32] noted that such injections increased the production of lactogen by the pituitary gland.

SUMMARY

When a female rat is united in parabiosis with a gonadectomized rat, the increased amount of follicle-stimulating hormone secreted in excess by the pituitary gland of the latter acts on the ovaries of the intact recipient partner. In the first stage there are enlargement of follicles and secretion of estrogen by the stimulated ovaries, with little effect on the mammary glands. In the second stage the estrogen secreted by the ovaries stimulates the pituitary gland of the recipient rat to release luteinizing hormone, with the resultant formation of corpora lutea in immature rats and enlargement of those present in mature rats, and to release also the factors causing hyperplasia of the mammary gland. In the third stage, the follicles of the ovaries continue to be stimulated and become cystic, but the corpora lutea disintegrate, presumably because estrogen has inhibited or exhausted the production of luteinizing hormone by the pituitary gland. There is at the same time active secretion by the hyperplastic mammary gland, presumably due to lactogen released by the pituitary gland. In the fourth stage the corpora lutea all disappear and the ovaries become composed of follicles and solid masses of granulosa cells. Copious secretion continues in the mammary gland, and in some rats two types of adenoma develop.

The pituitary gland shows loss of chromophil granules early, and as time passes it becomes very large and vascular and is composed entirely of chromophobe cells. Stunting of growth, lowering of blood sugar and failure of luteinization are attributed to hypofunction of the chromophil cells of the pituitary gland, which normally produce growth, diabetogenic and gonadotropic factors. Enlargement of the pituitary gland by hyperemia and hyperplasia are thought to be associated with hyperfunction of cells producing lactogen. Secretion by the mammary gland is not interfered with by apparent failure of formation of growth hormone and by absence of acidophil granules. Secretion by the mammary gland can occur in the presence of corpora lutea.

The experiments indicate that under the aforementioned conditions the follicle-stimulating hormone from the donor rat can cause the ovaries of the recipient rat to secrete estrogen in sufficient quantities to produce pathologic changes in the mammary and pituitary glands of the recipient. The changes produced in the pituitary gland are sometimes irreversible, as after separation of the rats the ovaries may remain entirely composed of granulosa cells and the pituitary gland may remain large and degranulated, presumably with failure of production of luteinizer. Such experiments represent a more biologic production of pathologic changes than those in which exogenous estrogen is intermittently injected.

30. Schooley, J. P., and Riddle, O.: Am. J. Anat. **62**:313, 1938.

31. Emge, L. A.: Surg., Gynec. & Obst. **68**:472, 1939.

32. Reece, R. P., and Mixner, J. P.: Proc. Soc. Exper. Biol. & Med. **40**:66, 1939.

Case Reports

SUDDEN DEATH WITH AN EXCESSIVE MYOCARDIAL CONCENTRATION OF EPINEPHRINE-LIKE SUBSTANCES IN A CASE OF OBESITY AND CYSTIC THYROID DISEASE

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In a previous report [1] a case of sudden, unexpected death in a young athlete was described in which the only significant alteration at postmortem examination was an excessive accumulation of epinephrine-like substances in the heart muscle. In that article reference was made to the literature on the colorimetric method employed and on experimental and clinical observations regarding the occurrence of acute heart failure and death due to excessive administration or secretory discharges of epinephrine and related cardiotoxic catechol compounds and their accumulation in the myocardium.

A similar case will be described here:

A 35 year old white housewife was admitted to a hospital April 24, 1944. The family history was insignificant except for epilepsy in one of the three sisters. As a child the patient had chickenpox and whooping cough. At the age of 20 she had measles. The tonsils were removed when she was 11. Menstruation began at the age of 14; it was always regular. She had two children, 12 and 9 years old. She had never undergone any serious illness. Three years prior to admission she was operated on for cystocele. At that time (1941) examination revealed normal conditions throughout except for marked obesity. The blood pressure was 126 systolic and 82 diastolic; the pulse rate was 80. The Eagle test gave a negative result. The urine contained no abnormal constituents. The white blood cell count was 11,700, with a normal differential count. She did not complain of any symptoms referable to the cardiovascular or the respiratory system.

After 1941 she began to notice increasing shortness of breath when bending her head forward—e. g., while shampooing her hair, or during sleep, when she used to snap her head back in order to breathe more easily. During the six months previous to the present examination she noticed gradual enlargement of the thyroid gland, which caused some difficulty in swallowing solid food; it also occasionally caused some difficulty in breathing in the recumbent position and on exertion, which was accompanied by wheezing. There were occasional dizzy spells in the morning, some palpitation on exertion but no edema, no cough, no tremors, no general nervousness, no loss of weight, no abnormal perspiration and no fainting spells. Pain in the chest was complained of only on the last day before she was admitted to the hospital.

On examination she showed obesity and diffuse enlargement of the thyroid gland but was in no apparent distress. The lungs and the heart were normal (findings not specified on the preliminary record). The pulse rate was 78; the respiratory rate, 20.

On April 25 after midnight a roommate of the patient noticed that she walked around to get a drink of water. Suddenly she clasped the upper part of her chest, called for a nurse and fell across the bed. There she was found gasping for breath and cyanotic. The pulse was irregular. One ampule of nikethamide was injected intramuscularly, and artificial respiration was given for twenty minutes without result. At 12:45 a. m. she was pronounced dead.

The autopsy was made by Dr. J. F. Gowdey, of the department of pathology of the University of Vermont. His condensed report follows:

"The body is well developed, obese, 69 inches (175 cm.) long. The weight is 250 pounds (113.5 Kg.). The skin, the ears, the nasal passages and the eyes appear normal.

"The heart weighs 400 Gm. The valves appear normal. There are no areas of softening, thinning or discoloration. The pulmonary artery is not remarkable. The myocardium shows a left ventricular thickness of 12 mm. and a right thickness of 2 mm. The coronary arteries are neither sclerosed nor narrowed. The orifices are patent. The aorta appears normal.

"The right lung weighs 550 Gm.; the left, 500 Gm. Both lungs appear mottled grayish and reddish with purplish bases. The sections show on pressure a moderate amount of frothy bloody fluid. The bronchi and bronchioli contain similar material.

"The spleen weighs 250 Gm. It is dark red and soft on the surface and on sections.

"The gastrointestinal tract, the gallbladder and the pancreas appear normal.

"The liver weighs 2,100 Gm. It is dark red, firm and smooth. The sections appear smooth and are mottled purplish and reddish.

"Each kidney weighs 150 Gm. They are smooth, firm and dark red. The capsule is stripped easily. The sections appear dark red; the pyramids, dark purple.

"The adrenal glands, the bladder and genitalia appear normal.

"The thyroid gland weighs 200 Gm. The lateral lobes are small and firm. They measure 7 by 3 by 1 cm. and 5 by 2 by 1 cm.; they are grayish on section. The isthmus is represented by a cystic ovoid reddish mass (8 by 7 by 4 cm.), which on section contains a grayish green soft gelatinous material and a small amount of thin watery fluid. The wall of this cystic structure is grayish and 7 to 8 mm. thick.

"The head was not examined.

"Microscopic examination of the lungs shows marked congestion of the alveolar capillaries. Many alveoli contain serum and some red cells. A number of alveoli contain one or two pigmented macrophages.

"The heart muscle is microscopically not remarkable. The large myocardial arterioles and a few of the smaller arterioles show mild intimal thickening and luminal narrowing.

"The liver shows mild cloudy swelling and mild to moderate congestion. Similar changes are present in the spleen and the kidneys. In the latter the congestion

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1. Raab, W.: Arch. Path. **36**:388, 1943.

is more pronounced. The renal vessels are not remarkable.

"Sections through the wall of the thyroid cyst show dense infiltration by lymphocytes and many small acini. In the thyroid gland there are a number of connective tissue septums and areas of loose connective tissue, densely infiltrated by numerous lymphocytes."

The anatomic diagnosis was: obesity; umbilical hernia; cyst of the isthmus of the thyroid gland and nonspecific thyroiditis; passive congestion of the lungs, the liver, the spleen and the kidneys of recent origin; pulmonary edema.

Twelve hours after death the heart muscle was examined for its content of epinephrine-like substances. A concentration of 1,249 color units per gram was found. The specific ratio was 1 : 1.22, indicating that a substantial part of this material consisted of epinephrine proper or sympathin.

In this case of clinically and anatomically unexplained sudden death the only objective finding apparently incompatible with survival and explanatory of cardiac failure without organic lesion was an abnormally high concentration of epinephrine-like substances in the myocardium as in another previously published case of sudden death.[1]

Analogous observations have been reported elsewhere [2] concerning the heart muscles of the majority of patients who had died in chronic or acute heart failure of hypertensive, arteriosclerotic or uremic origin or arising during the course of various fatal diseases.

In the case now reported no such primary disease existed. The cyst of the isthmus of the thyroid gland seemed to have interfered with respiration mainly through mechanical pressure on the trachea, although the postmortem observations suggested passive congestion of somewhat longer duration than the final attack of pulmonary edema.

Precordial pain, of which the patient complained shortly before her death, and pulmonary edema, with which she died, are typical results of either injection or secretory discharge of toxic amounts of epinephrine, e. g., in cases of paraganglioma.

No changes in the adrenal glands were observed in this case. On the other hand, the thyroid hormone is known to intensify the toxic effects of epinephrine on the heart. Thus it seems possible that the abnormal condition of the thyroid gland in this case may have contributed to the fatal effect of the epinephrine-like material in the heart muscle. In experiments on rats it had been found that an otherwise almost innocuous dose of epinephrine proved rapidly fatal through acute cardiac failure and pulmonary edema in animals pretreated with thyroxin.[3] The hearts contained concentrations of epinephrine-like substances above the critical level, which is about 2,000 color units per gram in rats.

Recently French and Dock [4] reported 80 cases of more or less sudden death among seemingly healthy young soldiers. Ten per cent of these men died during sleep; one of them awoke with pain before dying. Overweight was present in 91 per cent. Vigorous effort seemed to be a factor contributing to the fatal outcome in 50 per cent. None of the hearts were hypertrophic. All showed some degree of sclerosis of the coronary arteries but only few to a degree which in itself might be considered as directly fatal. It would seem rather that the changes in these arteries were not the immediate cause of death but merely the anatomic manifestation of repeated and prolonged exposure of the arteries and the heart to intense discharges of sympathomimetic amines, which are well known as exquisitely vasocardiotoxic agents.[5] Marked sclerosis of the coronary arteries can be produced in cholesterol-fed animals by prolonged enforced exercise,[6] which is accompanied by a considerable increase of epinephrine in the blood [7] and the heart. [2a]

Death can be assumed to have supervened in the cases of French and Dock as well as in the case now described and in previously published cases of sudden death as soon as the accumulation of cardiotoxic sympathomimetic amines in the myocardium exceeded the critical fatal concentration, which is about 1,000 color units per gram in man.

SUMMARY

Sudden, unexpected death occurred in a 35 year old woman with a cyst of the isthmus of the thyroid gland and nonspecific thyroiditis.

As in a previous case of sudden death of a young athlete, the only alteration observed at postmortem examination which seemed to be incompatible with survival and thus explanatory of the fatal outcome was an abnormal accumulation of epinephrine-like substances in the heart muscle.

2. Raab, W.: (*a*) Exper. Med. & Surg. **1**:188, 1943; (*b*) J. Lab. & Clin. Med. **29**:715, 1944.

3. Raab,[2a] pp. 198-199.

4. French, A. J., and Dock, W.: J. A. M. A. **124**: 1233, 1944.

5. Raab, W.: Arch. Path. **35**:836, 1943. Raab.[2]

6. Schmidtmann, M.: Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch. **5**:283, 1932.

7. Raab, W.: Exper. Med. & Surg. **1**:402, 1943.

AMEBIC COLITIS COMPLICATED WITH ABSCESS OF THE BRAIN

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Among the complications of amebic colitis, abscess of the liver is frequent. According to a tabulation by Simonds,[1] its incidence ranges from 3.48 per cent in surviving patients to 42.36 per cent in cases in which necropsies were performed. Involvement of the brain is a rare complication and is usually associated with similar involvement of the liver or of the lungs or of both. To date 61 cases of amebic abscess of the brain have been reported. Among these the first 2 without involvement of the liver or the lungs were reported by Kartulis,[2] the third by Putney and Baker [3] and the fourth by Stein and Kazan.[4] The fifth such case is herein recorded.

REPORT OF A CASE

A 54 year old white man was admitted to the University Hospitals, Oklahoma City, Feb. 20, 1944, in a semicomatose state. In 1940 he had amebic dysentery and was treated with repeated doses of emetine hydrochloride and carbarsone. The symptoms subsided for periods of several months. In February 1944 there was an exacerbation of symptoms, and on February 12, he was found on the street in a dazed condition. During the following twelve hours he complained of severe occipital pain. Two days later, although the headache persisted, he returned to work and continued at work until February 17, when he became mentally confused. On the following day he was admitted to Weedn Hospital, Duncan, Okla., where examination of the cerebrospinal fluid revealed 1,125 white blood cells per cubic millimeter with numerous fresh red blood cells. The red blood cell count was 5,200,000, and the white blood cell count was 34,500, with polymorphonuclears 87 and lymphocytes 13 per cent. Many motile amebas were seen in the stools. Meningococcic meningitis was suspected, sulfadiazine was administered and the patient was transferred to the Oklahoma City Detention Hospital. The therapy was continued for the next thirty-six hours without improvement, and he was transferred to the University Hospitals (service of Dr. Elmer R. Musick).

On admission the patient was semicomatose, delirious and dehydrated. There was marked opisthotonos with Kernig's and Brudzinski's signs. His blood pressure was 120 systolic and 80 diastolic; the pulse rate was 100 and the respiratory rate 24 per minute. There was slight exophoria of the left eye. There was no tenderness, rigidity or palpable mass in the abdomen. The deep tendon reflexes were diminished, with bilateral absence of knee and ankle jerks. There was an inconstant Babinski sign on the right. The optic disks were well defined, with no discernible changes. A definite tache cérébrale was noted. Roentgenologic examination of the skull revealed no abnormalities.

The urine was acid, with a specific gravity of 1.030; it contained a trace of albumin and no sugar. The red blood cell count was 4,100,000; the hemoglobin content was 13 Gm. per hundred cubic centimeters; the white blood cell count was 32,500, with polymorphonuclears 94 and lymphocytes 6 per cent. The cerebrospinal fluid contained 2,790 cells per cubic millimeter, predominantly polymorphonuclears with a few fresh red blood cells. The protein content was 70 mg. per hundred cubic centimeters; the globulin reaction was 4 plus. After settling, the supernatant fluid had a xanthochromic tint. No growth appeared on cultures in ten days. A few motile amebas were demonstrated in the feces.

Sulfadiazine and fluids were administered. By February 25 the patient's temperature dropped to 98.8 F., and he became rational. When he attempted to sit up in bed, he fell backward and to the right. No further evidence of a localized cerebral lesion could be discovered. On the following day an irregular spiking temperature developed, which varied from 100 to 104 F. The pulse rate remained about 60 per minute. He again became irrational and stuporous; the stiffness of his neck increased. A cisternal puncture on February 28 yielded a xanthochromic fluid containing small tissue fragments and clumps of white blood cells. No microorganisms were detected. The patient's condition became progressively worse, and he died on March 2, 1944.

The failure of response to sulfadiazine, the xanthochromic spinal fluid with tissue debris and the absence of demonstrable organisms suggested that the patient

From the Laboratories of the University Hospitals and the Department of Medicine, University of Oklahoma School of Medicine.

1. Simonds, J. P.: Quart. Bull. Northwestern Univ. M. School **17**:25, 1843.

2. Kartulis, S.: Zentralbl. f. Bakt. (Abt. 1) **37**:527, 1904.

3. Putney, F. J., and Baker, D. C., Jr.: Dis. of Chest **4**:20, 1938.

4. Stein, A., and Kazan, A.: J. Neuropath. & Exper. Neurol. **1**:32, 1942.

probably had an amebic abscess of the brain with rupture into the subarachnoidal space.

At necropsy (Dr. M. S. Terrell), two and one-half hours after death, the following pertinent data were recorded. The abdomen was sunken. There was a small amount of clear fluid in the peritoneal cavity. Both lungs were crepitant anteriorly and were firm and lumpy posteriorly. In the anterior portions the cut surfaces were pale pink; in the posterior portions the cut surfaces presented bulging red and gray areas. Mucopurulent material could be expressed from the bronchioles. The right lung weighed 760 Gm. and the left 450 Gm.

The liver measured 26 by 20 by 6.5 cm. and weighed 1,460 Gm. Its cut surfaces were uniformly red-brown.

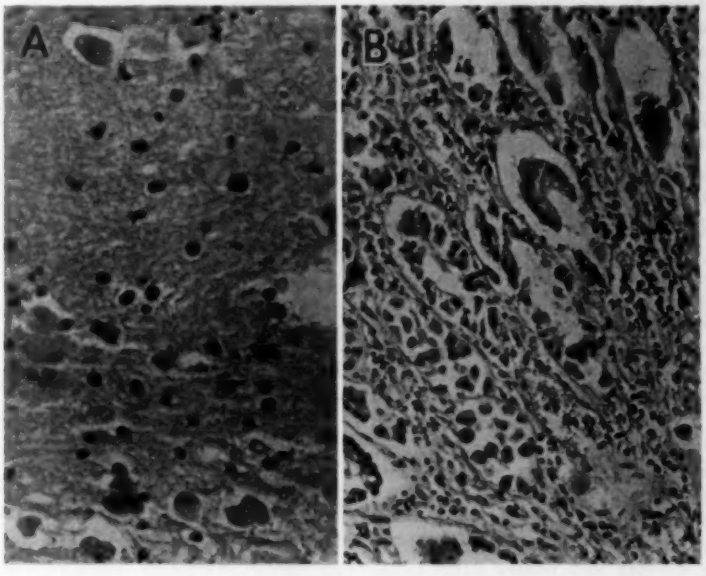
In the mucosa of the cecum and the proximal half of the ascending colon there were shallow ulcerated areas with yellow-gray bases and ragged edges, up to 1 cm. in diameter. Similar ulcers were seen in the rectum. There were no ulcers in the intervening portions of the colon.

The dura mater was tense. The leptomeninges were slightly cloudy. The cerebral convolutions were flattened and the sulci narrowed. There was a discolored, softened area in the right occipital lobe, 5 cm. in diameter. From a rent over this area about 100 cc. of red-brown creamy liquid escaped. The brain weighed 1,390 Gm. Further examination after fixation disclosed some asymmetry of the cerebral hemispheres. Their cut surfaces at the level of the optic chiasm had the usual pattern of gray and white matter. The cavities of the lateral and third ventricles were slitlike. There was red discoloration of their surfaces. At a level 3 cm. posterior to the optic chiasm, the posterior horn of the left ventricle was distended; the posterior horn of the right ventricle was collapsed. In the right occipital lobe there was a cavity 5 by 3 cm. with a ragged inner surface and tissue debris. In places it extended to the surface.

Organisms resembling Endamoeba histolytica were demonstrated in both the content of the cecum and the cavity in the occipital lobe of the brain.

The anatomic diagnosis based on the aforementioned observations was amebic colitis, amebic abscess of the right cerebral hemisphere and bronchitis and bronchiolitis with bilateral focal pneumonia.

Microscopically, the superficial layers of the mucosa of the cecum were desquamated. The cells lining the crypts were well preserved. In the tunica propria and filling the crypts of Lieberkühn, which were devoid of covering epithelium, there were oval or round bodies about three times the size of a white blood cell, stained blue and lavender. These bodies were seen scattered in the submucosa singly or in groups of two or three with a halo around them and no cellular reaction (fig., *B*). In some areas the mucosa was replaced by a fibrinopurulent exudate. The edges of the ulcer were overhanging, and amebas could be seen in the submucosa forming the base. In preparations from the rectum amebas were less conspicuous. No amebic lesions were seen in preparations from the liver and the lungs.



A, amebas in the brain substance near the abscess cavity; × 300. *B*, amebas in the crypts of Lieberkühn and in the submucosa of the cecum in a case of amebic colitis due to infection with Endamoeba histolytica; × 150.

In the leptomeninges covering the medulla oblongata and the pons varolii there was an infiltration with lymphocytes, plasma cells, large mononuclear cells and some polymorphonuclear granulocytes, which involved also the walls of blood vessels. In a preparation from the wall of the cavity one surface was bordered by a broad zone of necrotic debris infiltrated with similar cells. In the adjacent fairly intact brain tissue there were bodies stained light lavender, with a light halo around them, similar to those seen in the wall of the cecum (fig., *A*).

COMMENT

The mechanism by which E. histolytica reaches the brain is by no means clear, particularly in those rare instances in which, as here, the liver and the lungs are not involved. Stein and Kazan [4] suggested that the amebas reach the brain through the vertebral system of veins demonstrated by Batson.[5] This, however, does not explain why involvement of the brain is so rare and why it occurs late in the course of the disease.

5. Batson, C. V.: Ann. Surg. **112**:138, 1940.

SUMMARY

Amebic abscess of the brain occurred in a 54 year old white man with amebic colitis. This is the fifth case of amebic colitis in which abscess of the brain occurred without similar involvement of the liver and the lungs.

Notes and News

Registry of Veterinary Pathology.—Recently an arrangement was approved by the Surgeon General of the United States Army and the board of governors of the American Veterinary Medical Association for the establishment and maintenance at the Army Institute of Pathology, Army Medical Museum, Washington, D. C., of a Registry of Veterinary Pathology. This registry will become a unit of the American Registry of Pathology, an organization operating by the authority of the Surgeon General under the sponsorship of the National Research Council.

The purpose of the American Registry of Pathology is comprehensive investigation in certain special fields, which at present comprise: ophthalmic pathology, otolaryngologic pathology, orthopedic pathology, dental and oral pathology, neuropathology, dermatologic pathology, pathology of neoplasms, with special consideration of those of the endocrine glands, the kidney, the urinary bladder and the lungs. Through close cooperation with various national societies, records and material in these several specialties are brought together at the Army Institute of Pathology for systematic study. The number of specimens received is considerable; for example, there are now available for investigation 4,747 tumors of the urinary bladder and nearly 2,000 melanomas of human eyes. There are also on hand sections of eyes from many different species of animals. All the material and the records of the registry are available for study to graduate students and specialists, as well as other authorized persons.

For the Registry of Veterinary Pathology it is desired to assemble (*a*) material representing general pathologic anatomy, including vitamin deficiencies, specific diseases of different tissues and organs and examples of natural and experimentally induced neoplasia; (*b*) a complete collection of prepared slides representing the normal histology of the different species of animals, including domesticated and wild mammals, birds and cold-blooded vertebrates, and (*c*) material illustrating experimentally induced lesions of infectious diseases.

As material accumulates, loan sets of slides will be made available for study. Similarly, sets of lantern slides will be prepared which pertain to topics of special importance; these also will be available for loan to contributors.

This announcement is for the information of veterinarians and others interested in comparative pathology; it is hoped that they will make full use of the registry and send to it material deemed of interest for teaching and for the investigation of animal and human diseases. Material submitted should be addressed to: The Director, Army Institute of Pathology, Army Medical Museum (attention: Registry of Veterinary Pathology), Seventh Street and Independence Avenue, S. W. Washington 25, D. C. The director will be glad to furnish further instructions to contributors for submission of material to the Registry of Veterinary Pathology.

Medicolegal Conference and Seminar.—The Massachusetts Medico-Legal Society in conjunction with the medicolegal departments of the Boston medical schools has arranged for an all day conference at the Boston City Hospital Wednesday, Oct. 4, 1944. This meeting will be open to any physician, lawyer, police official, senior medical student or medical investigator who may care to register. There will be no fee. Notice of intention to attend should be sent prior to October 1 to Dr. W. H. Watters, Harvard Medical School, Boston.

The Harvard Medical School with the cooperation of the medical schools of Boston University and Tufts College offers a seminar in legal medicine October 2 to 7, inclusive. It is planned particularly for medical examiners and coroners' physicians but will be open to any suitable graduate. The course will consist of autopsy demonstrations, technic and interpretation of laboratory tests, study of the day by day cases of a medical examiner, round table conferences and the many subjects now included in the widening field of legal medicine. Enrolment has been limited to fifteen. The fee is $25. Application should be made on or before October 1 to Harvard Medical School, Courses for Graduates, 25 Shattuck Street, Boston 15.

General Reviews

SOME STATISTICAL ASPECTS OF CANINE TUMORS

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In the course of recent investigations on canine tumors a survey of the statistical aspects of neoplasms in dogs forcibly thrust into the foreground the small number of reports on this subject in the literature. Logically suggested was a summarizing of these papers in an attempt to shed some light on the problem, to evolve methods of enhancing the understanding of the incidence of canine neoplasms, at least so far as the type and the location of the tumors and the age, the sex and the breed of the dogs might be concerned, and to stress the importance of tumors in dogs to the vast field of comparative oncology.

REVIEW OF THE LITERATURE

Among 702 dogs on which autopsies were made at the Pathologic Institute of the Faculty of Veterinary Medicine of the University of Berlin, Casper [1] found 51 with carcinoma, the lesions being distributed as follows: breast, 9; liver, 7; intestinal glands, 6; prostate, 4; thyroid gland, 4; testes, 3; pleura and pericardium, 3; lungs, 3; kidneys, 2; ovary, vagina, tail, anus, skin, uterus, pancreas, spleen, bronchial lymph nodes and submaxillary lymph nodes, 1 each.

In a long paper on the statistical aspects of the occurrence and the surgical treatment of tumors in dogs, Fröhner [2] analyzed the data on several hundred tumors seen between 1886 and 1894 at the Hospital Clinic and the Polyclinic of the Faculty of Veterinary Medicine of the University of Berlin. At the Hospital Clinic 643 tumors were noted in 8,999 sick dogs. At the Polyclinic 2,228 tumors were observed in 61,474 sick dogs. Of the 643 tumors operated on at the Hospital Clinic, 576 were diagnosed as follows: 262 as carcinoma, 97 as fibroma, 65 as papilloma, 44 as sarcoma, 39 as lipoma, 17 as vaginal polyp, 16 as mucous cyst, 14 as struma, 9 as ranula, 7 as atheroma, 4 as corneal dermoid and 2 as angioma. In 1893 and 1894 at the Polyclinic 229 canine tumors included 214 with the following classification: carcinoma, 83; fibroma, 33; mammary chondroma, 27; papilloma, 20; mucous polyp, 17; sarcoma, 15; corneal dermoid, 6; mucous cyst, 5; dermoid cyst, 2; struma, 2; ranula, 2; lipoma, 1; angioma, 1. Between 1886 and 1894 at the Polyclinic 1,484 tumors diagnosed as carcinoma, fibroma and sarcoma were observed. The most frequent sites of carcinoma at both clinics were the skin, the breast and the anus. The less frequent or uncommon sites of carcinoma were the prostate, the testis, the vagina, the penis and the thyroid gland. The author never saw carcinoma in dogs less than 2 years old. Of the 65 dogs operated on for this type of cancer by the author, 10 were 2 to 4 years old, 18 were 5 to 6, 22 were 7 to 8, 12 were 9 to 10, and 3 were 12 to 13. He described 70 dogs with carcinoma, 21 with sarcoma, 23 with fibroma, 20 with papilloma and 12 with lipoma with regard to the location of the growth and the breed, the age and the sex of the animal.

The 39 lesions of the skin diagnosed as carcinoma were located chiefly in the eyelids, the ears, the back, the base of the tail, the legs, the prepuce and the scrotum of male dogs. The ages were 2 to 5 years in 11 dogs, 6 to 10 in 23, and 11 to 15 in 5. The 31 cancers of other sites diagnosed as carcinoma were located chiefly in the breasts and the anus of 12 male and 19 female dogs. Of 18 mammary cancers diagnosed as carcinoma, 1 occurred in the penultimate breast of an 8 year old male pointer. The ages were 2 to 5 years in 5 dogs, 6 to 10 in 23, and 11 to 15 in 3.

The 21 cancers classified as sarcoma were located in the alveolar arches, the palate, the gums, the lymph nodes, the bones, the breasts, the cheeks and the legs. In 20 of the 21 dogs with sarcoma, 17 males and 3 females were represented, and the ages were 2 to 5 years in 12 and 6 to 10 years in 8.

The 23 tumors listed as fibroma were situated in the chest, the extremities, the back, the tail, the eyelids, the ears and the breasts of 22 males and 1 female. The ages were 1 to 5 years in 13 dogs, 6 to 10 in 9, and 11 to 15 in 1.

The 20 tumors recorded as papilloma were found in the eyelids, the ears, the extremities, the chest, the buttocks and the shoulders of male

From the Department of Pathology, University of Colorado School of Medicine.

1. Casper: Arch. f. Thierh. **19**:14, 1893; cited by Casper, M.: Ergebn. d. allg. Path. u. path. Anat. **3** (pt. 2):754, 1896.

2. Fröhner: Monatsh. f. prakt. Thierh. **6**:1, 79 and 111, 1895.

dogs. The ages were 1 to 5 years in 14 dogs, 6 to 10 in 5, and 11 to 15 in 1.

The 12 tumors classified as lipoma were located in the extremities, the buttocks, the back, the anus, the shoulders and the breasts of male dogs. The ages were 1 to 5 years in 4 animals, 6 to 10 in 6, 11 to 15 in 1, and 16 in 1.

In summary, of 143 tumors diagnosed as carcinoma, sarcoma, fibroma, papilloma and lipoma, 59 occurred in dogs 1 to 5 years old, 73 in dogs 6 to 10 years old, and 11 in dogs 11 to 15 years old. Of 145 dogs with tumors, 122 were males and 23 were females. The breeds represented were as follows: bulldog, 39; pug, 16; Leonberger, 15; pointer, 15; poodle, 14; dachshund, 11; cart dog, 5; terrier, 5; Doberman pinscher, 5; St. Bernard, 4; Newfoundland, 4; hound, 4; seidenspitz, 3; sheep dog, 2; spitz, greyhound and boxer, 1 each.

McFadyean [3] reported 23 cases of canine carcinoma. In 5 of these the cancer was located in the skin; in 5, in the anus; in 3, in the breast; in 3, in the liver; in 2, in the prepuce; in 2, in the kidneys; in 1 in the pharynx, and in 1, in the orbit.

In a long statistical review Sticker [4] discussed cancer as observed in (*a*) dogs treated at the Hospital Clinic and the Polyclinic of the Faculty of Veterinary Medicine of the University of Berlin, (*b*) dogs examined by Schütz at the Pathologic Institute in Berlin and by Jöhne at the Institute of Pathology of the Veterinary Academy at Dresden and (*c*) dogs whose cases had been reported in the literature. Between 1886 and 1901, 15,455 dogs were treated at the Hospital Clinic in Berlin; 465 were affected with cancer. Between 1893 and 1901 at the Polyclinic in Berlin 75,818 dogs were seen; 491 were affected with cancer. In 604 dogs from both institutions, the known sites of origin of cancer included the breast (299), the skin (143), the anus (78), the penis (14), the testis (9), the scrotum (6), the eyelids (6), the vagina (4) and the bladder, the ureter, the gum and the parotid gland (1 each). In 70 of these dogs with external cancer the ages were 2 to 3 years in 10, 5 to 6 in 18, 7 to 8 in 20, 9 to 10 in 14, 11 to 12 in 4, and 12 to 15 in 4.

Between 1886 and 1901 autopsies were made on 1,306 dogs at the Pathologic Institute in Berlin; 72 of these had carcinoma. Of 82 primary lesions diagnosed as carcinoma in the dogs examined by Schütz at this institution, the breasts contained 23, the prostate 10, the kidneys 9, the liver 9, the testes 7, the lungs 5, the thyroid gland 4, the skin 3, the anus, the urinary bladder and the pleura 2 each and the ureter, the ovary, the uterus, the vagina, the abdominal cavity and the mesenteric lymph nodes 1 each. Of 48 dogs in Berlin which died or were killed because of cancer, the ages were 2 to 4 years in 4, 5 to 6 in 5, 7 to 8 in 11, 9 to 10 in 17, 11 to 12 in 7, and 13 to 15 in 4.

Of the 60 dogs with carcinoma examined by Jöhne at the Institute of Pathology in Dresden, 24 had the primary lesion in the thyroid gland, 8 in the skin, 6 in the liver, 5 in the breast, 5 in the kidneys, 3 in the mesenteric lymph nodes, 2 in the anus, 2 in the lungs and 5, respectively, in the bladder, the testis, the ovary, the uterus and the bronchial lymph nodes.

From the literature Sticker [4] collected 61 cases of primary carcinoma. In 14 of these the lesion was in the breasts; in 7, in the anus; in 6, in the skin; in 6, in the kidneys; in 4, in the liver; in 4, in the thyroid gland; in 3, in the lungs; in 2, in the orbit; in 2, in the prepuce; in 2, in the bladder; in 2, in the vagina; in 2, in the pancreas; in 7, respectively, in the ureter, the testis, the ovary, the parotid gland, the pharynx, the nasal cavity and the heart. The ages in 15 dogs were 2 to 4 years in 1, 5 to 6 in 3, 7 to 8 in 3, 9 to 10 in 1, 11 to 12 in 1, and 13 to 15 in 6.

Cadiot [5] in four years saw 22,450 dogs, of which 854 had neoplasms—an incidence of 3.8 per cent. He mentioned that in 1858 Urbain Leblanc (basing his conclusions on hundreds of personal observations and on reports of 62 cases of cancer in animals in veterinary journals) stated that the organs affected most commonly by cancer in the dog and the cat were the breast, the testis, the penis and sheath, the prostate, the uterus, the vagina, the rectum, the liver, the spleen, the lungs and the lymph nodes and that the course of cancer in these animals was more rapid than in herbivores.

Jöhne [6] observed 209 canine tumors between 1878 and 1903 at the Pathologic Institute of the Dresden Veterinary University. Of these 209 neoplasms, 16 were diagnosed as fibroma, 4 as leiomyoma, 3 as myxoma, 3 as chondroma, 2 as lipoma, and 1 as osteoma.

Murray [7a] found 16 neoplasms of the mammary tissues and 14 of the extremities (the latter grouped as sarcoma) among 64 dogs with cancers. He [7b] tabulated 26 neoplasms diagnosed as sarcoma and 23 diagnosed as carcinoma occur-

3. McFadyean, J.: J. Comp. Path. & Therap. **12**:137, 1899.

4. Sticker, A.: Arch. f. klin. Chir. **65**:616 and 1023, 1902.

5. Cadiot, J.: Rec. de méd. vét. **84**:5 and 87, 1907.

6. Jöhne, cited by Casper, M.: Ergebn. d. allg. Path. u. path. Anat. **11** (pt. 2):1068, 1907.

7. Murray, J. A.: (*a*) Vet. J. **64**:621, 1908; (*b*) in Third Scientific Report, Imperial Cancer Research Fund, London, 1908, pp. 41-60.

ring in dogs. Those classed as sarcoma were distributed as follows: legs, 11 (6 in the forelegs, 5 in the hindlegs); breast, 5; palate, 2; intestine, 2; lip, ear, scalp, neck, body wall and tail, 1 each. Of the 23 classed as carcinoma, 8 were located in the breasts, 5 in the pharynx, 3 in the anus and 7 in the palate, the tongue, the buccal epithelium, the skin, the foreleg, the liver and the rectum, respectively. The types of sarcoma were stated to be round cell (10 cases), spindle cell (1), alveolar (1), mast cell (1), melanotic (1), osteosarcoma (1) and miscellaneous (4). In 2 cases the sarcoma was not typed. The types of carcinoma were given as squamous cell (11 cases), adenocarcinoma (6), untyped (3) and miscellaneous (3). Among the 49 dogs with cancerous neoplasms the ages of 12 were stated; 2 were under 6 years, 4 were 6 to 10 years, and 6 were 11 to 15 years old. The sex of 21 dogs was recorded; 16 were females and 5 were males. Two 12 year old males were afflicted with mammary neoplasms, one with adenocarcinoma and the other with osteosarcoma.

In 1912 and 1913 Joest[8] observed several interesting tumors at the Institute of Pathology of the Veterinary Academy in Dresden. Of 111 dogs on which autopsies were made in 1912, 4 had carcinoma of the thyroid gland, with metastases to the lung in 3, and 5 had, respectively, thymoma, fibrosarcoma of the thorax with extension to the pleura, round cell sarcoma of the intestine, spindle cell sarcoma of the abdominal wall and carcinoma of the pancreas with metastases to the liver. The thymoma, which occurred in an 11½ year old female pointer, caused compression atelectasis of the lungs, extended to the base of the heart, obstructed the thoracic duct and produced bilateral chylothorax. Among 136 dogs on which autopsies were done in 1913, he encountered the following: 5 dogs with carcinoma of the thyroid gland, 1 of which had metastases in the lung; an 11 year old female spitz with adenomyomatosis of the uterus; an 8 year old male Newfoundland with adenoma of the hypophysis; 7 dogs with, respectively, spindle cell sarcoma of the left kidney, multiple lymphosarcoma of the intestine, carcinoma of the frontal sinus with extension to the nasal cavity, adenocarcinoma of the right kidney, adenocarcinoma of the liver with metastases in the lungs, multiple fibroma of the uterus and lymphatic leukemia.

In a lengthy review of tumors in animals Fölger[9] compiled the types of tumors seen in dogs between 1896 and 1911 at the Hospital Clinic and in the Polyclinic of the Faculty of Veterinary Medicine of the University of Berlin. At the Hospital Clinic 1,124 tumors were observed in 14,569 dogs, an incidence of 7.8 per cent. Of the 602 tumors which were definitely diagnosed, 236 were diagnosed as carcinoma, 101 as fibroma, 99 as papilloma, 56 as sarcoma, 52 as lipoma, 29 as corneal dermoid, 20 as adenoma, 3 as keloid, 3 as myxoma, 2 as hemangioma and 1 as melanosarcoma. At the Polyclinic 3,581 tumors were noted in 129,740 dogs, an incidence of 2.8 per cent. Of the 1,937 neoplasms definitely diagnosed, 529 were recorded as carcinoma, 474 as adenoma, 385 as papilloma, 319 as fibroma, 43 as corneal dermoid, 41 as sarcoma, 39 as chondroma, 39 as lipoma, 19 as keloid, 5 as hemangioma, 2 as melanosarcoma, 1 as myxoma and 1 as osteoma.

From the laboratory of veterinary pathology and bacteriology at the University of Pennsylvania, Crocker[10] recorded 53 tumors observed in 1,548 autopsies on dogs between 1909 and 1919, an incidence of 3.4 per cent. Among these 53 tumors 34 were diagnosed as carcinoma, 8 as sarcoma, 4 as papilloma, 2 as fibroma, 2 as osteoma and 3 as chondroma, adenoma and leukemia, respectively. Of the 34 classed as carcinoma, 16 were located in the breast, 3 in the duodenum, 3 in the prepuce, 3 in mixed tumors, 2 in the jejunum, 2 in the testes, 2 in the thyroid gland, 1 in the rectum, 1 in the liver and 1 in the kidney.

Of the 8 neoplasms diagnosed as sarcoma, 2 were situated in the genitalia, and 1 each in the hard palate, the vagina, the mandible, the scapula, the humerus and the neck. Of the tumors diagnosed as papilloma, 2 were located in the prepuce and 2 in the vagina; of those diagnosed as fibroma, 1 occurred in the uterus and 1 in the perineum; of those classed as osteoma, 1 was in the first lumbar vertebra and 1 in the penile bone. The chondroma was in a lung, and the adenoma was in the small intestine.

Feldman[11a] observed 17 canine tumors. Of these 4 were diagnosed as lymphosarcoma, 3 as adenoma, and 10, respectively, as fibrosarcoma, lipoma, endothelioma, myxofibroma, melanosarcoma, lymphocytoma, embryonal carcinoma, carcinoma, adenocarcinoma and papilloma. The primary sites of 28 tumors in dogs seen by him[11b] were the vagina in 2 cases of lymphosarcoma, the thyroid gland in 2 cases of adenocarcinoma with metastases to the lungs, the inner canthus of the eye in 2 cases of adenoma and

8. Joest, E.: Ber. ü. d. Königl. tierärtzl. Hochschule zu Dresden **7**:67, 1912; **8**:75, 1913.

9. Fölger, A. F.: Ergebn. d. allg. Path. u. path. Anat. **18**:372, 1917.

10. Crocker, W. J.: Cornell Vet. **9**:142, 1919.

11. Feldman, W. H.: (*a*) Am. J. Path. **2**:545, 1926; (*b*) J. Cancer Research **11**:436, 1927; (*c*) Proc. Staff Meet., Mayo Clin. **3**:253, 1928.

the skin or the hypodermis in 6 cases of lymphosarcoma, 2 cases of carcinoma and 1 case of fibrosarcoma. In addition there were, among the 28 primary tumors, single examples of myxosarcoma of the mediastinum, subcutaneous lipoma of the hip, subcutaneous endothelioma of the thoracic wall, generalized lymphocytoma of lymph nodes, embryonal carcinoma of the testis, carcinoma of the pharynx, adenoma of the breast, sebaceous adenoma of the ear, papilloma of the lip, leiomyoma of the cecum, melanosarcoma of the breast with metastases to the brain and spinal cord, malignant teratoma (This occurred in the ovary of an old Chesapeake Bay dog and weighed 20½ pounds [9 Kg.]), and multiple nodular "endothelioma" or "mesothelioma" of the lungs and pleurae (this in an adult mongrel). Among 41 canine tumors Feldman [11e] found the following types: carcinoma in 12, lymphosarcoma in 11, adenoma in 6, papilloma in 2, leiomyoma in 2 and lipoma, myxofibroma, lymphocytoma, malignant endothelioma, melanoepithelioma, mesothelioma, malignant teratoma and fibrosarcoma in 1 each.

Winkler [12] listed over 50 source references for the subject of canine oncology in his long review of the comparative pathology of tumors. The articles referred to were largely concerned with single cases or with various special phases of neoplastic diseases in dogs and contained no statistical data not already surveyed in the present communication.

Auler and Wernicke [13] classified 585 canine tumors observed at the Wernicke Clinic between 1920 and 1931. Among these 585 tumors 173 were grouped as sarcoma, 153 as carcinoma, 70 as adenoma, 60 as "benign round cell sarcoma," 31 as cystadenoma, 25 as lipoma, 15 as fibroma, 15 as myoma, 12 as adenofibroma, 12 as myxoma, 10 as chondroma, 3 as fibromyoma, 2 as lipofibroma, 1 as hemangioma and 1 as melanocytoblastoma. Of the 336 examined histologically 320 were checked by Blumenthal, of the Institute for Cancer Research of the University of Berlin, who designated 60 as "benign round cell sarcoma," 56 as mixed cell sarcoma, 52 as carcinoma, 50 as sarcoma, 38 as mixed cell carcinoma, 26 as adenoma, 11 as cystadenoma, 9 as fibroma, 8 as venereal or Sticker's lymphosarcoma, 6 as cancroid, 5 as adenofibroma, 5 as myxoma, 2 as myoma and 5 as fibromyoma, lipofibroma, lipoma, chondroma and hemangioma, respectively. "Benign round cell sarcoma," was described as a circular, flat, smooth, hairless, firm, light red nodule in the cutis, looking like a button, never growing larger than a 25 cent piece, never forming metastases and encountered nearly always in young dogs after the age of 6 months. Following etching with toughened silver nitrate, cauterization and extirpation, the tumor is brought to easy and final healing. Histologically the tumor consists of round and spindle cell sarcoma. In spite of the histologic picture, the tumor was observed to be absolutely harmless in over 60 cases.

The 585 tumors were distributed in various locations as follows: the skin (fibroma, sarcoma, myxoma, fibrosarcoma, melanosarcoma, fibrocarcinoma, carcinoma and cancroid); the subcutaneous tissue (myxoma); the gums and cheeks (sarcoma, melanosarcoma and carcinoma); the tongue (melanocytoblastoma); the palate (sarcoma and fibrocarcinoma); the salivary glands (carcinoma); the anal pouch (adenoma and carcinoma); the perineum (myoma); the vagina (fibroma, myoma, adenoma, fibromyoma, sarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma and carcinosarcoma); the breast (adenoma, adenofibroma, sarcoma, osteosarcoma, fibrosarcoma, angiosarcoma, adenocarcinoma, melanocarcinoma, carcinoma and cancroid); the penis (lymphosarcoma); the testis (sarcoma and carcinoma); the lymph nodes (sarcoma); the thyroid gland (carcinoma).

In addition to 35 tumors diagnosed as infectious sarcoma, 20 in the penis and 15 in the vagina, Mendoza [14] tabulated 38 canine tumors, 17 located in the skin, 15 in the breasts and 6 in other sites. The tumors of the skin included the following types: hygroma (4 cases), carcinoma (4), squamous cell carcinoma (3), fibrosarcoma (2), lymphosarcoma (2), adenocarcinoma (1) and lipoma (1). The tumors of the breast included carcinoma (8 cases, 1 with osseous metaplasia), adenocarcinoma (3) and colloid carcinoma (1), fibrosarcoma (1), sarcoma (1) and sebaceous cyst (1). The remaining 6 neoplasms were diagnosed as adenocarcinoma of the ear, sarcoma of the spleen, fibrosarcoma of the vagina, myofibroma of the uterus, seminoma and fibroma, respectively.

Antoine, Liégeois, and Verstraete [15] reported a series of 214 dogs with tumors examined in 1933. Some of these animals had one and others several (as many as 5 or 6) tumors. In 189 cases the nature of the tumor was diagnosed by microscopic examination, in most cases after removal of the tumor and in some cases after biopsy. In 25 cases the nature of the tumor was

12. Winkler, K.: Ergebn. d. Biol. **5**:692, 1929.

13. Auler, H., and Wernicke: Ztschr. f. Krebsforsch. **35**:1, 1931.

14. Mendoza, M. A.: Bol. Liga contra el cáncer **9**:1, 1934.

15. Antoine, Liégeois and Verstraete: Bull. Acad. roy. de méd. de Belgique **14**:301, 1934.

diagnosed by clinical characteristics, since operation or biopsy was refused by the owner of the dog. Of 209 cases in which the age was recorded, it was 1 to 5 years in 32, 6 to 10 years in 96, 11 to 15 years in 78 and 16 to 18 years in 4. In 206 cases the sex was known; it was female in 133 and male in 73. The frequency of tumors in the four breeds with the highest rates of tumor incidence as compared with the total numbers of each seen in consultation was 2.03 per cent in the fox terrier, 2.27 per cent in the Brussels griffon, 2.39 per cent in the German shepherd and 3.16 per cent in the Groenendal shepherd. The following breeds were represented: mongrel (45 dogs), fox terrier (33), Brussels griffon (16), Groenendal shepherd (13), German shepherd (13), dwarf spaniel (8), French bulldog (7), loulous (7), Tervueren shepherd (6), water spaniel (6), lap dog (6), leash dog (5), cocker spaniel (5), shepherd (4), English setter (4), boxer (4), German basset hound (4), Pekingese (4), Mechlin shepherd (3), Korthals griffon (3), bouvier des Flandres (2), stable griffon (2) and St. Bernard, German bulldog, Beauce shepherd, Dutch shepherd, Scotch shepherd, pointer, French brach, German brach, Gordon setter, Airedale terrier, Irish terrier, whippet, schipperke, and pinscher (1 each).

The following types of epithelial cancers were found: carcinoma (76 cases), adenocarcinoma (23), squamous cell carcinoma (11), basal cell carcinoma (7), sarcocarcinoma (2), atypical epithelioma (2) and cylindric epithelioma (1). The ages at which carcinoma occurred in these 122 cases were: 1 to 5 years in 8, 6 to 10 in 44, 11 to 15 in 65, 16 to 18 in 3 and not known in 2.

The following types of sarcoma were observed: spindle cell (16 cases), round cell (14), fibrosarcoma (10), myxosarcoma (6), osteosarcoma (3) and lymphosarcoma (2). The ages at which sarcoma occurred in these 51 cases were: 1 to 5 years in 8, 6 to 10 in 23, 11 to 15 in 11, 16 to 18 in 2, and not known in 7.

The following types of benign tumors were seen: fibroma (15 cases), cystadenoma (12), adenoma (9), chondroadenoma and osteoadenoma (9), lipoma (6), sebaceous cyst (6), fibromyoma (4), osteoma (4), fibroadenoma (3), papilloma (3), fibrolipoma (2) and adenomyoma, myxoma, myxofibroma, angioma and myxomyoma (1 each). The ages at which the tumor occurred in these 78 cases were: 1 to 5 years in 15, 6 to 10 in 31, 11 to 15 in 20, and not known in 12.

The breasts were involved by 127 tumors, 5 located in the first breasts, 18 in the second, 13 in the third, 36 in the fourth and 42 in the fifth; 13 were not definitely located. The types of tumors found in the breasts were: carcinoma (51 cases); adenocarcinoma (24); cystadenoma (12); chondroadenoma and osteoadenoma (9); adenoma (4); spindle cell sarcoma (4); lipoma (3); basal cell carcinoma, fibroadenoma, round cell sarcoma, myxosarcoma, fibroma, osteoma and sarcocarcinoma (2 each); adenomyoma, fibrosarcoma, fibrolipoma and angioma (1 each).

The skin was the site of 66 tumors, including the following types: squamous cell carcinoma (10 cases); spindle cell sarcoma (8); carcinoma (6); round cell sarcoma (6); sebaceous cyst (6); fibrosarcoma (5); basal cell carcinoma (5); lipoma (3); adenocarcinoma, myxosarcoma, fibroma, osteoma and myxofibroma (2 each); atypical epithelioma, cylindric epithelioma, adenoma, fibroadenoma, osteosarcoma, fibrolipoma and myxoma (1 each).

The vagina was the location of: fibroma (6 cases); fibromyoma (3); round cell sarcoma, spindle cell sarcoma and fibrosarcoma (2 each); myxosarcoma, lymphosarcoma and myxomyoma (1 each).

The following types were noted in the anus: carcinoma (14 cases), adenocarcinoma, squamous cell carcinoma and round cell sarcoma (1 case each).

The types located in the gum were osteosarcoma (2 cases), papilloma, fibroma and spindle cell sarcoma (1 case each).

The testes were the site of carcinoma in 4 cases and of round cell sarcoma in 1 case.

Carcinoma was observed in the lip in 2 cases; atypical epithelioma and fibroma in 1 case each.

Carcinoma, adenocarcinoma and papilloma were seen in the eyelids, in 1 case each; carcinoma in the thyroid gland in 4 cases; adenoma in the "corps clignotant" in 3 cases; round cell sarcoma in the penile sheath in 2 cases; osteosarcoma in the humerus in 1 case; lymphosarcoma in the spleen in 1 case and papilloma in the external ear in 1 case.

COMMENT

The data presented in the papers on the statistical aspects of canine neoplasms do not allow any accurate quantitative analysis since the statistical results were not uniform; statistics in some of the early papers overlapped; the nomenclature varied and was often not specific; some series were based on autopsies and others on surgical material, the latter type being preponderant; and statistics were incomplete in that some series did not include comprehensive data on age, sex and breed of dogs and types and sites of neoplasms encountered.

In spite of these drawbacks, some general statements may be formulated. The most common

age group among dogs afflicted with both cancerous and noncancerous tumors is that between 6 and 10 years. The next most common age group is that of 11 to 15 years and the least common is that of 2 to 5 years so far as cancerous neoplasms are concerned; this order is reversed in regard to noncancerous neoplasms. The very presence of tumors in dogs is frequently dependent on their survival into old age, usually 6 years and beyond, although probably the oldest average age which dogs attain under ideal conditions is between 11 and 15 years. Cases are on record of dogs living beyond 20 years of age, but they are rare. Since most dogs live in cities, the great majority are fortunate to live until they reach the age group of 6 to 10 years, because distemper, the automobile and poisoning take a high toll.

The sex of dogs afflicted with tumors apparently depends somewhat on the tastes of dog owners in the country from which a given series of canine neoplasms might be selected; i. e., Germans prefer males (Fröhner [2]) and Belgians prefer females (Antoine and associates [15]). In the United States the taste in canine pets seems to run toward males.

No conclusive data could be gathered concerning breed, although Fröhner [2] and Antoine and associates [15] showed that bulldogs, pugs, shepherds, fox terriers, dachshunds, griffons, setters, pointers, hounds, poodles and spaniels rank high on the list of dogs affected with neoplasms.

Dogs are more likely to be afflicted with cancerous than with noncancerous neoplasms. Carcinoma is more common than sarcoma. Among the more usual noncancerous types are fibroma, adenoma, papilloma, lipoma and leiomyoma. Dermoid of the cornea is common and chondroma is frequent because of the large number of dogs that have chondroma of the breast. Rarer noncancerous types are myxoma, osteoma and angioma.

Superficial and readily accessible neoplasms are most commonly found in dogs, involving prominently the breasts, the skin, the subcutaneous tissue, the anus, the mouth, the penis and the vagina. This observation may be largely dependent on the far fewer routine autopsies as compared with the surgical removals or biopsies of external neoplastic lesions. To a lesser degree, limitations of veterinary diagnostic facilities and surgical skill in the diagnosis and treatment of internal canine cancer may play a role. Among the most common locations for internal cancer in the dog are the liver, the prostate, the thyroid gland, the testes, the lymph nodes and the lungs. Since the dog is not subject to the same vicissitudes of evolution as man, although environment cannot be ruled out as a causative factor, a lower incidence of internal cancer in dogs may eventually be incontrovertibly proved when large series of dogs on which autopsies have been made in the canine cancer age become available.

Because the last informative statistical report on canine tumors appeared in 1934,[15] the recording of the surgical and autopsy experience of various large centers dealing with sick dogs might be encouraged, especially in the United States, so that an adequate picture of the incidence of canine neoplasms could be gained, at least so far as the types and the location of tumors and the age, the sex and the breed might be concerned. Veterinarians and workers in other branches of comparative oncology should make an effort to perform routine, reasonably complete autopsies on dogs afflicted with tumors. This would involve education of the public to the point where the people would at least be as willing to consent to autopsies on dogs as to grant permission for necropsies on human beings. Another helpful step would be the establishment of a canine tumor registry, not only for the accurate recording of statistical data, especially data from veterinary institutions of various types, but also for the registration of tumors of specific types and locations, as in the bone tumor and thoracic tumor registries for the recording of human neoplasms. The nomenclature of neoplasms in man and animals could be more standardized and comprehensible than in the past, so that the diagnoses of all workers in comparative oncology might be uniform and mutually interchangeable. Through such measures the value of canine neoplasms to comparative oncology could be more generally appreciated and fully understood. The natural high incidence of spontaneous tumors in dogs, which are usually carefully observed because of their value as pets, would be likely to yield information of substantial aid in the solution of cancer in animals and, most important of all, in man.

Book Reviews

The Pathogenesis of Tuberculosis. By Arnold R. Rich, M.D., associate professor of pathology, Johns Hopkins University School of Medicine. Price, cloth, $10.50. Pp. 950, with 35 page index, 89 figures, 20 tables and 1,417 references. Springfield, Ill.: Charles C Thomas, Publisher, 1944.

The purpose of this work is to present the principles and the factors that determine the character, the progression and the regression of tuberculous lesions.

The culture, the chemical composition and the type characteristics of the tubercle bacillus are discussed. The evidence in favor of a pathogenic filtrable form of the organism is considered insufficient to be convincing, as are also the attempts to correlate morphologic aspects and virulence. Although nothing is known of any type-specific carbohydrate, and though there is little information as to any qualitative differences in the lipids of virulent and avirulent strains, and despite a lack of evidence that virulence is determined by the proteins of the bacillus, there probably are chemical and physical variations between virulent and avirulent tubercle bacilli. Pathogenesis depends partially on metabolic potentialities of the different strains and types, and dissociation in vivo may occur clinically. No searching study of the relation of the enzymes of the bacillus to virulence has been made.

There is an interesting and critical presentation of studies of native resistance in which variations of that factor corresponding to species, race and individual constitution are pointed out. Native resistance is not due just to a rapid development of acquired resistance. Differences in metabolic demands of the various organs may create varying degrees of competition between them and the bacillus for nutrient materials. The tension of available oxygen in organs perhaps has an important part in the survival of the tubercle bacillus and may be a factor in the efficacy of collapse therapy.

The differences between anaphylaxis and hypersensitivity such as that shown to tuberculin are listed, and the factors now known which control the development of the altered states are discussed. The local damage of tissue in tuberculosis is not due to any appreciable toxin but results from the altered response of the cells of the host to the bacillus and its breakdown products. Hypersensitivity does not necessarily parallel resistance, and there is no evidence now available, in the opinion of the author, that hypersensitivity in the absence of acquired resistance can either inhibit the distribution of or destroy pathogenic bacteria; nor is acquired resistance dependent on it. He concludes that at present hypersensitivity may be regarded in some instances as decidedly deleterious, in other instances as exerting no appreciable harmful or beneficial effect and in some cases as possibly an auxiliary of acquired forces of resistance.

He theorizes on the bilateral involvement of adrenal glands and eyes as due to an antigen-antibody reaction with organ specificity. There is perspicacious argument for considering the mechanism of acquired resistance in tuberculosis as similar to that in other infections; opinion to the contrary is considered based on a lack of evidence and not on positive contradictory observations. No cellular or humoral mechanism is known that destroys tubercle bacilli apart from mononuclear phagocytes; though any specific enhanced capacity for phagocytosis depends on the presence of antibody. There is no acceptable evidence that fixed tissue cells acquire any ability to resist infection independently of phagocytes and antibodies.

Arguments for and against exogenous and endogenous reinfections are presented, with the conclusion that either may occur and that evidence does not support any dogmatic attitude in favor of one.

In chapter 20 the principles of pathogenesis are applied to the descriptions and interpretations of lesions seen at autopsy. Evidence is quoted that tuberculous meningitis and pleural effusions do not result directly from the blood stream disseminating the organisms but from caseous foci discharging the organisms into the cerebrospinal fluid and into the pleural space respectively.

This book is a most valuable contribution to medical literature. It is replete with observations presented clearly and lucidly that are pertinent to the principles and mechanisms of any infection. All who are interested in tuberculosis will find a careful perusal of its pages most profitable and pleasant. It is a critical perspective of the entire picture of the pathogenesis of tuberculosis, wide in scope, penetrating in analysis, stimulating, provocative, exhaustive in facts and conservative in conclusions.

Infectious Anemias Due to Bartonella and Related Red Cell Parasites. By David Weinman, parasitologist to the 1937 Harvard Expedition to Peru and instructor in comparative pathology and tropical medicine at Harvard University Schools of Medicine and Public Health. Transactions of the American Philosophical Society, vol. 33, pt. 3. Price, $1.25. Philadelphia: The American Philosophical Society, Transactions 33, Part 3, 1944.

Besides the preface (Tyzzer) and the introduction there are four chapters. The first chapter summarizes in detail the history and the knowledge of human bartonellosis. Depending apparently on the response to infection with Bartonella bacilliformis, described by Barton in 1905, this infectious disease appears in two forms: Oroya fever, a febrile anemia, which may occur in epidemic form, and verruga peruviana, a cutaneous eruption, as was demonstrated by Carrión in 1886, who died from Oroya fever after inoculating himself with verruga material, hence the name Carrión's disease. The distinctive cellular lesions of Oroya fever were described by Strong and Tyzzer. The limitation of bartonellosis to Andean regions along the western part of South America corresponds to the distribution of species of sandflies (Phlebotomus) which are known to transmit the disease to human beings. In the course of his comprehensive and scholarly review on bartonellosis, Weinman neglects no opportunity to point out the many problems that await solution, e. g., the lack of effective means of prevention and of treatment of Oroya fever, the death rate of which in epidemics may be 40 per cent and higher. The name Oroya fever comes from the epidemic which occurred in 1870 during the construc-

tion of the railroad from Lima to Oroya, Peru, and which is estimated to have taken 7,000 lives—"every crosstie in the railroad represented a human life." The second and third chapters deal with blood infections in animals (animal bartonellosis and eperythrozoonosis) more or less similar to human bartonellosis and caused by organisms that "should fall within the domain of the bacteriologist." The fourth chapter is devoted to the public health aspects of bartonellosis. Here are considered the epidemics, the present extent of the disease, the epidemic factors—the human and insect hosts and Bartonella bacilliformis—and the possibilities of the disease spreading. The present methods of controlling bartonellosis are not adequate, and the situation requires continued careful study. Weinman's monograph is a highly important contribution to the literature on bartonellosis. It is "a reliable, modern, and full source of information, and reference; the South American literature, which is so difficult of access, being particularly well represented."

Books Received

HEART DISEASE. By Paul Dudley White, M.D., lecturer in medicine, Harvard Medical School; physician to the Massachusetts General Hospital, Boston. Third edition, completely revised and reset. Pp. 1025, with 138 illustrations. Price $9. New York: The Macmillan Company, 1944.

ARTIFICIAL PNEUMOTHORAX IN PULMONARY TUBERCULOSIS, INCLUDING ITS RELATIONSHIP TO THE BROADER ASPECTS OF COLLAPSE THERAPY. By T. N. Rafferty, M.D., formerly resident physician, William H. Maybury Sanatorium (Detroit Municipal Sanatorium), Northville, Mich. Pp. 192, with 26 illustrations. Price $4. New York: Grune & Stratton, Inc., 1944.

THE NEUROSURGICAL PATIENT: HIS PROBLEMS OF DIAGNOSIS AND CARE. By Carl W. Rand, clinical professor of neurological surgery, University of Southern California School of Medicine, Los Angeles. Pp. 576, with 121 illustrations. Price $4. Springfield, Ill.: Charles C Thomas, Publisher, 1944.

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